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Artefactual Mitral Regurgitation: Source of Diagnostic Error

Experimental Behavioral Control

Preoperative Irradiation: A Review

Abstracts

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ARTEFACTUAL MITRAL REGURGITATION: A SOURCE OF DIAGNOSTIC ERROR DURING LEFT VENTRICULAR CINEANGIOCARDIOGRAPHY

Armando Susmano
Stanley J. Heller
Richard A. Carleton

Cardiac catheterization and angiocardiography are well established procedures. However, these techniques occasionally provide factitious data or data which are misinterpreted. In turn, these may lead to faulty conclusions and to diagnostic errors. Factitious mitral insufficiency[1] has recently been described as a result of a cardiac catheter placement between the ball and seating ring of a prosthetic mitral valve. Phenomena of this type are not infrequent and are only detected by critical analysis of data and correlation with clinical findings.

The present brief report concerns an infrequent, but easily detected, source of artefactual mitral regurgitation and the features which led to its identification.

CASE REPORT:

Mrs. D. B., a 50-year-old white housewife, was admitted to Presbyterian-St. Luke's Hospital for evaluation of chest pain. She had led a vigorous, athletic life until substernal chest pain during exertion developed eight months before. In addition, the patient gave a history of rheumatic fever at age eight and of a heart murmur ever since.

The physical examination was entirely normal except for a blood pressure of 160/90 mm Hg and a Grade III/VI systolic ejection murmur heard maximally at the base, but with radiation to the carotid arteries and to the cardiac apex.

The electrocardiogram was abnormal but not diagnostic of prior myocardial infarction. Chest x-rays were normal. Cardiac catheterization and coronary arteriography were performed to evalu-

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ate the coronary arteries and to exclude significant valvular disease.

The studies revealed normal intracardiac pressures except for a left ventricular pressure of 177/13 mm Hg and an abnormally high cardiac output at 5.5 liters per minute per square meter. The right coronary artery was normal. Both the left circumflex and anterior descending arteries were small, but smooth and unobstructed. No pressure gradient across the aortic valve was found and no left-to-right shunts were demonstrated by indicator dilution curves. Left ventricular cineangiocardiography was performed. Fifty milliliters of Hypaque 50% was injected through a #7 French catheter with six radially arrayed side holes. As shown in frames enlarged from the 35-millimeter cine film (50 frames per second), prompt left atrial opacifications occurred (Fig. 1) with extension of the contrast agent back to the pulmonary veins (Fig. 2). No arrhythmias were observed during the cineangiocardiogram.

The observed mitral regurgitation, in association with a small left coronary artery, led to an initial conclusion of mitral regurgitation probably due to papillary muscle dysfunction.

COMMENT:

The clinical diagnosis of mitral insufficiency is at times difficult. It has been well recognized that the auscultatory features of mitral insufficiency may mimic those of aortic stenosis and vice versa.[2,3] Ejection-type murmurs have been described in mitral regurgitation, especially when due to ruptured chordae or to papillary muscle dysfunction.[4,5] Thus, a murmur of the quality described in the patient reported could have had either an aortic or mitral valvular origin.

The patient described had a small, narrow left coronary artery. Thus the combination of an atypical murmur, a

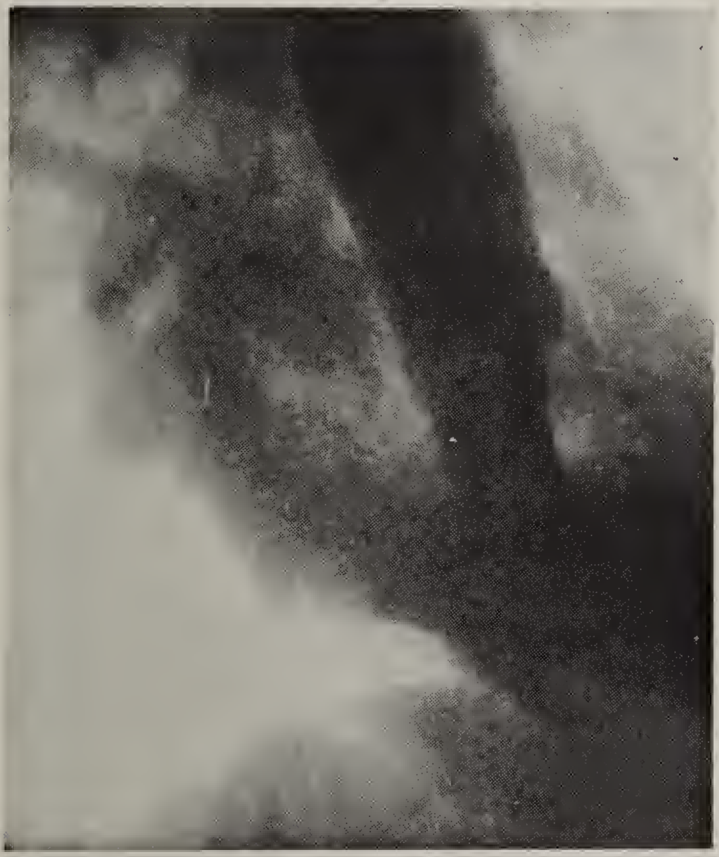

Fig. 1—Faint left atrial opacification is present after left ventricular injection. However, sequential analysis of cine frames disclosed that the opacification began in early ventricular diastole.

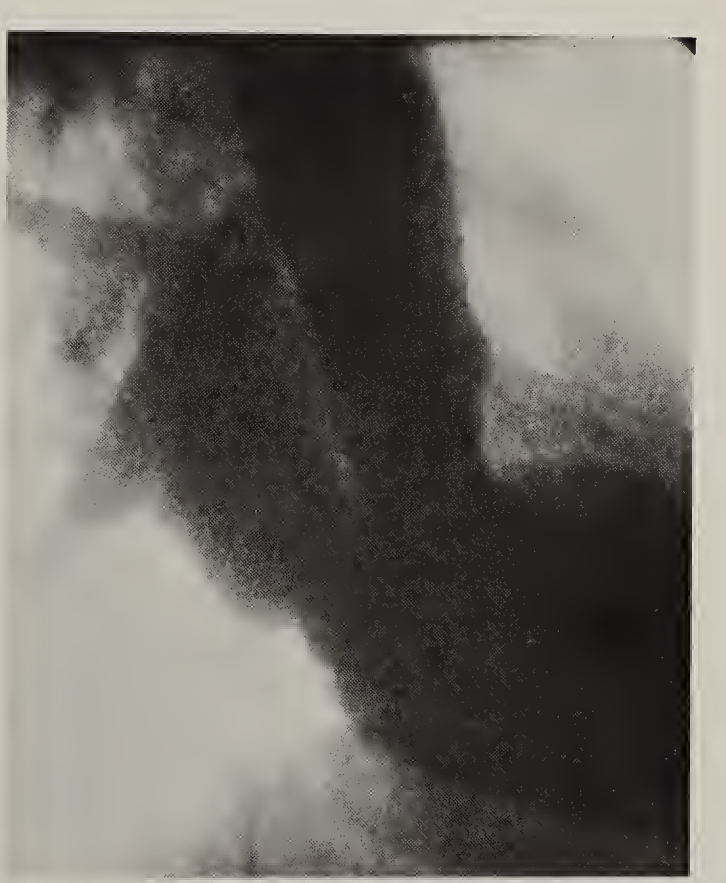

Fig. 2.—By late diastole, intense atrial and pulmonary venous opacification had occurred.

small coronary artery, and a moderately severe mitral regurgitation led to an initial diagnostic synthesis of coronary disease with papillary muscle scarring and dysfunction producing the murmur and the marked regurgitation.[6]

Alternatively, it seemed possible that functional transient mitral regurgitation could develop in the presence of coronary artery disease during the diagnostic studies.[7]

However, subsequent analysis showed these conclusions to be erroneous. A critical review of the cineangiogram demonstrated several features which eliminated the error.

Firstly, the side-hole catheter used for the pressure injection of contrast material was so oriented prior to injection (Fig. 3) that one or more jets were directed into the mitral orifice during diastole, although the catheter was clearly in the left ventricular cavity. Secondly, left atrial and pulmonary venous opacification appeared and increased only during ventricular diastole (Figs. 1 and 2). Thirdly, the pulmonary veins, which had opacified late during ventricular diastole, had contrast material washed back into the left atrium by unopacified blood during ventricular systole (Fig. 4). It was the latter, easily recognized event which provided the initial clue to the error in diagnosis.

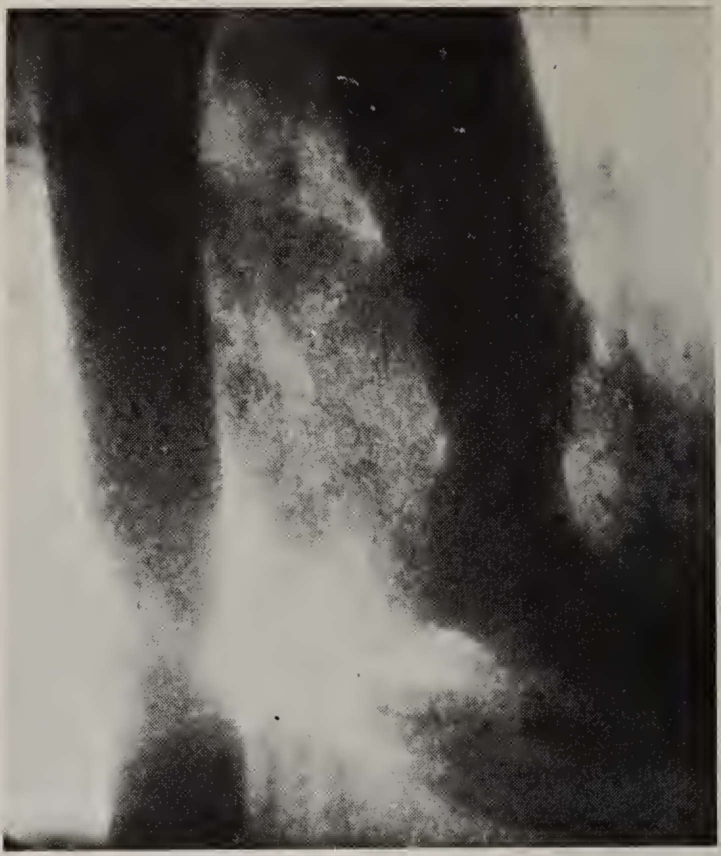

Fig. 4.—By the end of the left ventricular systole which occurred after the injection had ceased, the pulmonary veins were no longer opacified; only a minimal amount of dye remained in the left atrium despite continued left ventricular opacification. The descending aorta is now opacified.

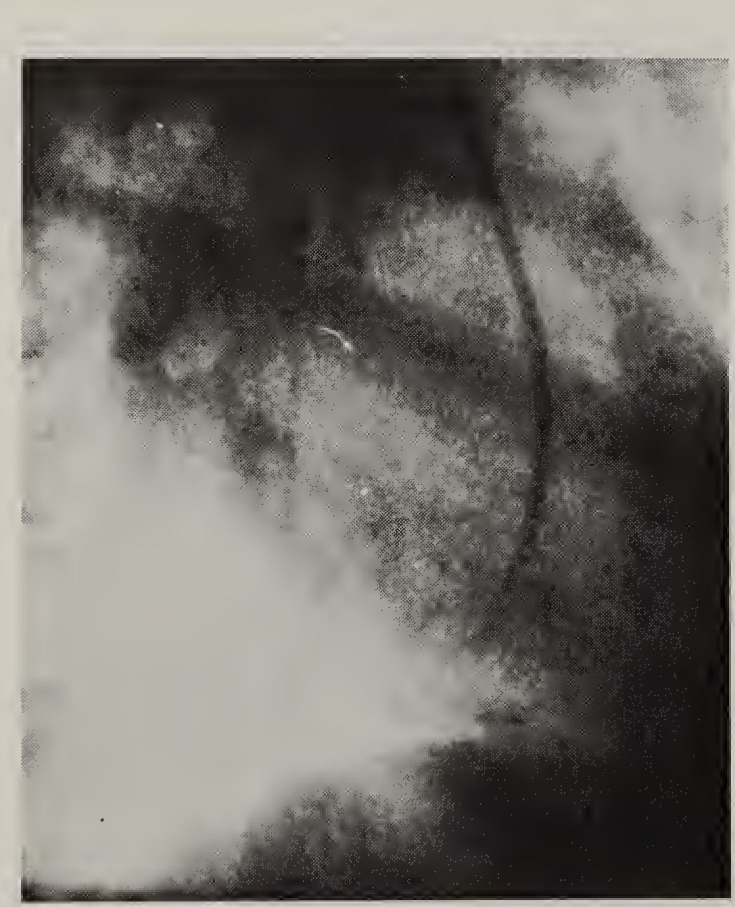

Fig. 3.—Before injection, the catheter was inside the left ventricular cavity with the side holes directed toward the mitral valve orifice.

The lesson of this diagnostic pitfall is clear: pathologic mitral regurgitation is an event occurring during ventricular systole. Detection of left atrial opacification alone does not suffice for the diagnosis.

SUMMARY

A patient with a systolic murmur, dominant right coronary artery and a small left coronary artery underwent diagnostic studies which revealed mitral regurgitation during selective left ventricular cineangiocardiography. This raised the diagnostic possibility of papillary muscle dysfunction.

Critical analysis of the cineangiocardiograms revealed the regurgitation to be artefactually produced during ventricular diastole. The initial clue to this finding was the emptying of contrast material from the pulmonary veins during ventricular systole.

Mitral regurgitation is a hemodynamic phenomenon of ventricular systole; artefactual diastolic mitral regurgitation should be excluded to establish the diagnosis.

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BEHAVIORAL CONTROL BY A STIMULUS THAT PREDICTS REINFORCEMENT

Derek P. Hendry

A thoroughly studied parameter of the learning process is delay of reinforcement, or the time between a response and a reinforcer. As this time increases, all modern studies agree that the reinforcing effect rapidly diminishes. The rate of decline in reinforcing effectiveness varies, however, from study to study, and this has been attributed to the variability in the degree to which the response is strengthened by conditioned reinforcement, which would, indeed, be expected to vary from study to study. The popularity of this interpretation strongly depends on studies by Perin[1] and Grice[2] who attempted to minimize conditioned reinforcing effects. The main results of these studies are shown in Fig. 1.

Perin's study used rats in a Skinner box containing a lever that could be moved laterally in either direction. After the rats were trained to push the lever in either direction, they were reinforced for pushing it in the non-preferred direction. When the correct response occurred, the lever silently withdrew from the box and a food reward was delivered after delays (for different groups of 25 rats) of 0, 2, 5, 10, 20 and 30 sec. Each rat could maximize his food rewards by changing from the preferred response to the now-correct non-preferred response. The rate with which this change occurred is essentially what is plotted in Fig. 1. No point is plotted for a delay of 30 sec. because 9 of the 25 rats failed to maintain responding with so long a delay of reinforcement. Removal of the lever after the correct response was an important detail, because it prevented "extra" responses and ensured a uniform delay, and because it prevented visual cues of the lever being present during reinforcement and thereby acquiring conditioned reinforcing value through classical conditioning. However, suppose the rat "froze" in the position of the last (correct) response, producing a distinctive pattern of proprioceptive cues until the delivery of reward; these proprioceptive cues would then become reinforcing by association with reward, and the rat would be immediately reinforced when it later adopted the same posture. Thus it would be immediately reinforced for the correct response on the next trial even though the primary reinforcer were delayed. If we assume that "traces" of proprioceptive cues exist, it is not necessary to assume that the rat actually "froze," because the traces would be associated with reinforcement. This argument, fantastic as it seems, was used by Spence[3] to explain why any learning at all occurred with delay of reinforcement. It also determined the design of

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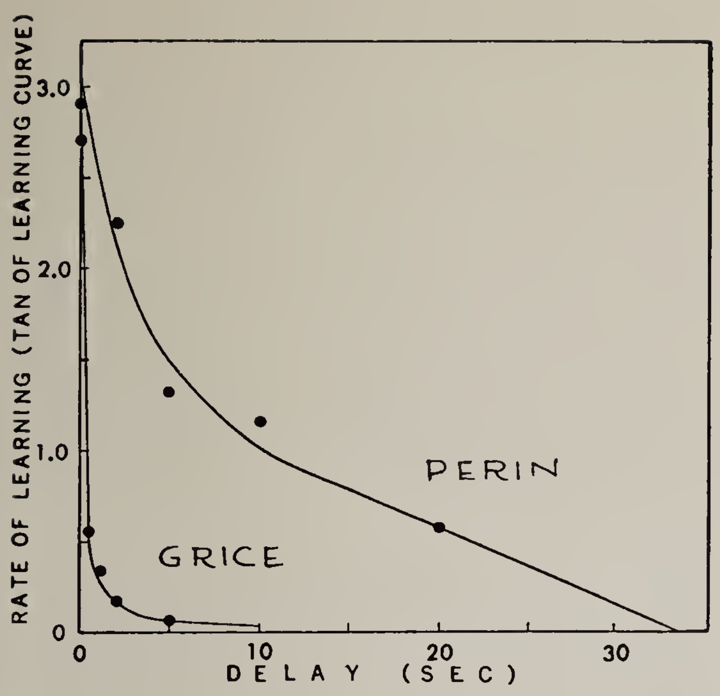

Fig. 1.—Speed of learning as a function of delay of reinforcement obtained by Perin (1943) and Grice (1948). After Grice (1948).

Grice's[2] study. Grice's rats had a choice between a black or white compartment which led to a grey alley ending in a grey goal box where they were rewarded with food. The black (or white) compartment always led to reward, while the white (or black) never did. The delay of reinforcement for the initial choice was controlled (at 0, 0.5, 1.2, 2.0, 5.0 and 10.0 sec.) by varying the length of the grey alley or by detaining the rat in the alley. The rate of learning to choose the correct compartment is what is plotted in Fig. 1. Since the left-right *position* of the correct alley varied from trial to trial, association of proprioceptive traces with reward would not lead to a correct initial choice.

These results have led to a position in behavior theory that is difficult to reconcile with common sense or with other biological phenomena. It is essentially that the organism has no "permanent" memory and that all learning and remembering is mediated by some remote association process within the organism. This is one general implication of the position that the action of reinforcement is immediate and confined to the instant of reinforcement itself. When rewarded, the rats in Perin's[1] study could not "remember" the correct response they had made 30 sec. previously. When rewarded, the rats in Grice's[2] study could not "remember" that they had 5 sec. earlier entered a black compartment. The extent to which they could perform correctly with shorter delays was due, we are asked to believe, only to the nature of the relation between the situation at the moment of choice and the situation at the moment of reinforcement.

The implausibility of this position encourages a consideration of other interpretations of the results of Perin and Grice. Perin's animals initially were more likely to make the wrong (*W*) response rather than the right (*R*) response. From his results it is clear that the animals at first emitted a series of responses, *W*, *W* . . . *W*, *R*, and that, as expected, the number of *W* responses was gradually reduced on successive trials. The rate of reduction of the *W* responses is proportional to the rate of learning shown in Fig. 1. However, the emission of many *W* responses represents inefficient performance rather than positive evidence for the absence of learning. On the other hand, the failure of some animals to keep responding if the reward were dealyed for 30 sec. shows that the general conditions of the experiment, in this case, including notably the initial probability of the correct response, were inadequate to sustain performance. Had the animals kept responding they could, perhaps, have shown the development of a preference for the correct response.

If Grice's rats had ignored the brightness difference in the first compartment, they would be reinforced on a random 50% of runs. This condition sustains performance almost as well as 100% reinforcement.[4] Therefore, we can interpret Grice's results thus: The small incentive difference between 50% and 100% reinforcement will appear in measures of behavioral preference but is attenuated by delays between choice and reinforcement, until with a delay of about 10 sec. the incentive values are essentially equivalent. Note that this interpretation does not preclude the animal's remembering for 10 sec. if the differential consequences were greater in incentive value, or, as we might say, if remembering were worthwhile.

A previous experiment showed that stimuli associated with definite periods of time before reinforcement were themselves reinforcing.[5] In that experiment the time elapsed in a fixed-interval (FI) schedule of reinforcement on one response-key was indicated by the stimulus that could be produced on a second, distinctive, observing-response key. The maintenance of observing behavior showed the reinforcing effect of stimuli that preceded reinforcement for up to 6 min. Observing responses did not increase the frequency or probability of reinforcement. The subjects (pigeons) did not confuse the two keys, as shown by the distinctive performances on each key. The stimuli that signified a long delay of reinforcement were reinforcing despite the fact that these stimuli were never closely associated with reinforcement. Observing behavior gradually disappeared when only one stimulus was produced by the observing response, which showed that observing behavior was not maintained by the mediation of proprioceptive (postural) stimuli, as referred to in the above discussion of Perin's and Grice's results.

A possible interpretation of the results described above is that responses will be reinforced by stimuli which predict the occurrence of a biologically important event, such as food for a hungry organism. It is plausible that such a predictive stimulus will be effective only to the degree that other predictive stimuli are not available. We have to assume that the animal is not able to predict the occurrence of reinforcement so accurately without the exteroceptive predictive stimuli. In other words, we suppose that the animal is in a state of "uncertainty" which is reduced by the predictive stimulus.

From this point of view, the perceptual apparatus of the organism may be regarded as a mechanism for the identification of sequences of stimuli terminating in biologically important stimuli. The behavior of the organism is instrumental in exposing, clarifying and facilitating access to these sequences of stimuli. The phenomenon of conditioned reinforcement is one implication of this view. Such a view simplifies our understanding of delay-of-reinforcement experimental procedures. In these procedures the sequences presented to the animal are always of the type:

$$S_x \longrightarrow S_o \longrightarrow S^R$$

where:

S_o is a uniform situation, such as the interior of a Skinner box, preceding both S^R and no-S^R.

S_x is a distinctive event, usually an operant response and/or its effects.

S^R is a reinforcement.

A successful organism will detect that S^R was preceded by a stimulus sequence that included S_x, and responses that produce S_x will be reinforced, thus raising the probability that the organism will be brought into the presence of the reinforcer.

From this point of view the typical experimental procedures for investigating the effect of delay of reinforcement on strength of responses and on strength of a conditioned reinforcer are essentially the same, differing only in what brings about the event S_x. In the former case the experimenter specifies that S_x is an operant response (or its immediate consequences), and in the latter case S_x is an experimenter-controlled exteroceptive stimulus. For example, in a study by Jenkins[6] S_x was a 3-sec. buzzer, and S_o was a simple delay period. The reinforcing effect of the buzzer was later tested.

In summary, in Perin's, Grice's and Jenkins' experiments the sequences terminating in reinforcement were respectively:

$S_x \longrightarrow$	$S_o \longrightarrow$	S^R
Pushing-lever-in → Skinner-box	Skinner-box- → without lever	food
Entering-black → compartment	grey alley →	food
Skinner-box → with-Buzzer	Skinner-box- → without-Buzzer	food

When S_o lasts a relatively long time, the reinforced and non-reinforced sequence will differ in many ways because of the variable, uncontrolled behavior of the animal during S_o. Such variation will make the detection of the reinforced sequence more difficult, which could account for the delay-of-reinforcement effect in studies where S_o is simply a blank period of time interposed between response and reinforcement. The absence of an attenuation of reinforcing effect by delay of reinforcement in the experiments of Hendry and Dillow[5] may be due to the fact that S_o was filled with schedule-controlled behavior.

The present experiment was undertaken to examine the pigeons' detection of a predictive stimulus and its behavioral effect as a function of the duration of S_o, the time between the predictive stimulus and the reinforcer. The general method was to make available a 1 min. response-produced stimulus in a 5.5 min. fixed-interval schedule of reinforcement (FI 5.5), and systematically to vary the position of the stimulus.

METHOD

The subjects were three male adult white Carneau pigeons. Birds 1 and 2 had no other experimental history. Bird 3 had been exposed to schedules of reinforcement similar to those described. They were maintained at 75-80% of free-feeding weight. Standard experimental chambers were used, having a feeder, an extractor fan which also provided masking noise, a light for general illumination, and two keys 3 inches apart which could be lit from behind with various colored lights.

After a brief session of training to peck a white key for food, the birds were placed directly on the complete schedule described below, with one session of 40 reinforcements each day and occasional extra sessions in lieu of extra feeding in their cages.

Different contingencies were programmed on each key. One key was called the "food" key because pecks on it sometimes produced food. The other was called the "observing" key because pecks on it sometimes produced a stimulus temporally related to the availability of food. This is called a predictive, or timing, stimulus. Every 5.5 min. (or longer) a peck on the food key would produce a 4-sec. presentation of mixed grain (FI 5.5). The magazine was lit by a white 2-watt light during reinforcement. The predictive stimulus became available for an observing response in the 5th min., or 4 min. after the last reinforcement. It remained available for 1 min., and when turned on, stayed on until the end of the minute. This condition is denoted as $T=4$., T was varied from 4 through 0, performance being stabilized at each value in turn.

The food key was lit with a white light continuously, except during reinforcement, when it was dark. The predictive stimulus was a red light which appeared on the observing key; at other times the observing key was orange. These stimulus conditions were chosen to minimize any possible confusion between the food key and the observing key. The left/right position of the keys was changed after every reinforcement.

RESULTS AND DISCUSSION

The initial performance of Birds 1 and 2 is shown in Fig. 2. Responding on the observing key occurred at a very low rate for most of the first 30 or 40 sessions, before reaching the appreciable rate (about 10 responses per min.) sustained for the bulk of the experiment. A very low rate on the observing key is to be expected inasmuch as responses on it

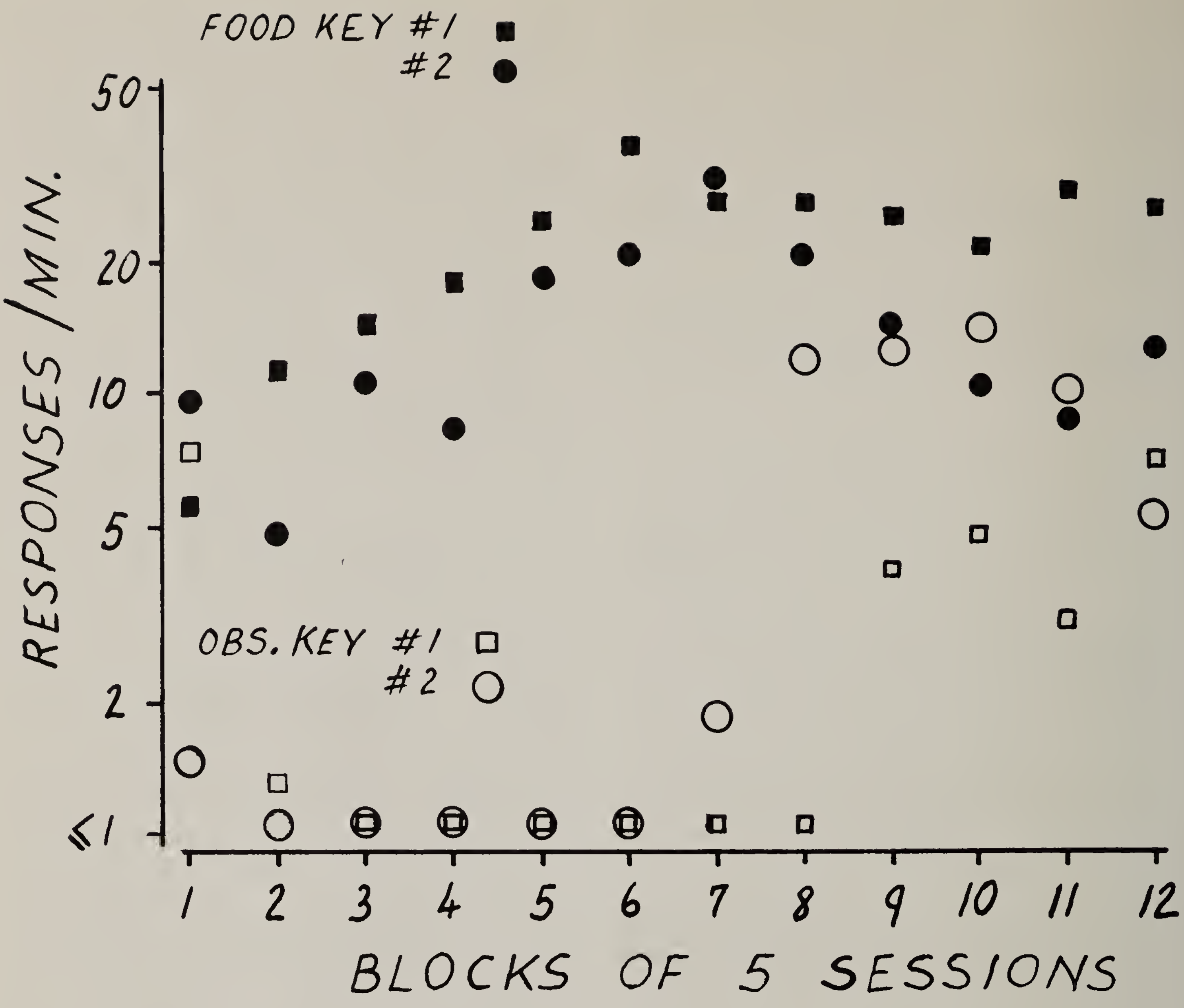

Fig. 2.—Birds 1 and 2. Response rate on Food key and Observing key during the first 60 sessions of the experiment. In these sessions T = 4, that is, a response on the Observing key 4-5 min. since the last reinforcement produced a stimulus on the keys for the rest of that minute.

never produced primary reinforcement. The fact that an appreciable rate on the observing key did develop is evidence for a conditioned reinforcing effect of the observed stimuli. This is confirmed by the fact that when responses on the observing key had no consequences, at the end of the experiment, every bird's rate on the observing key again dropped to a very low value, less than 1 response per minute. A respresentative performance from the initial sessions, when the fixed-interval had taken effect but before observing behavior had developed, is shown in Fig. 3. The positively accelerated response rate between reinforcements is typical of fixed-interval schedules.[7]

Fig. 4 shows typical final performance of Bird 2 at T = 4 (at T = 4 the predictive stimulus, when obtained, went off 30 sec. before reinforcement became available.) Fig. 4 illustrates the uniformity of performance obtained with Bird 2 from one interval to the next on each key. It is clear that both the primary reinforcer and the predictive stimulus affected responding on each key, though apparently not in the same way. The pattern of responding on each key for the other two birds was roughly similar.

It is difficult to compare response rates on the two keys from cumulative records such as Fig. 4, especially when response rates are very low. Numerical measures of performance are given in Figs. 5, 6,

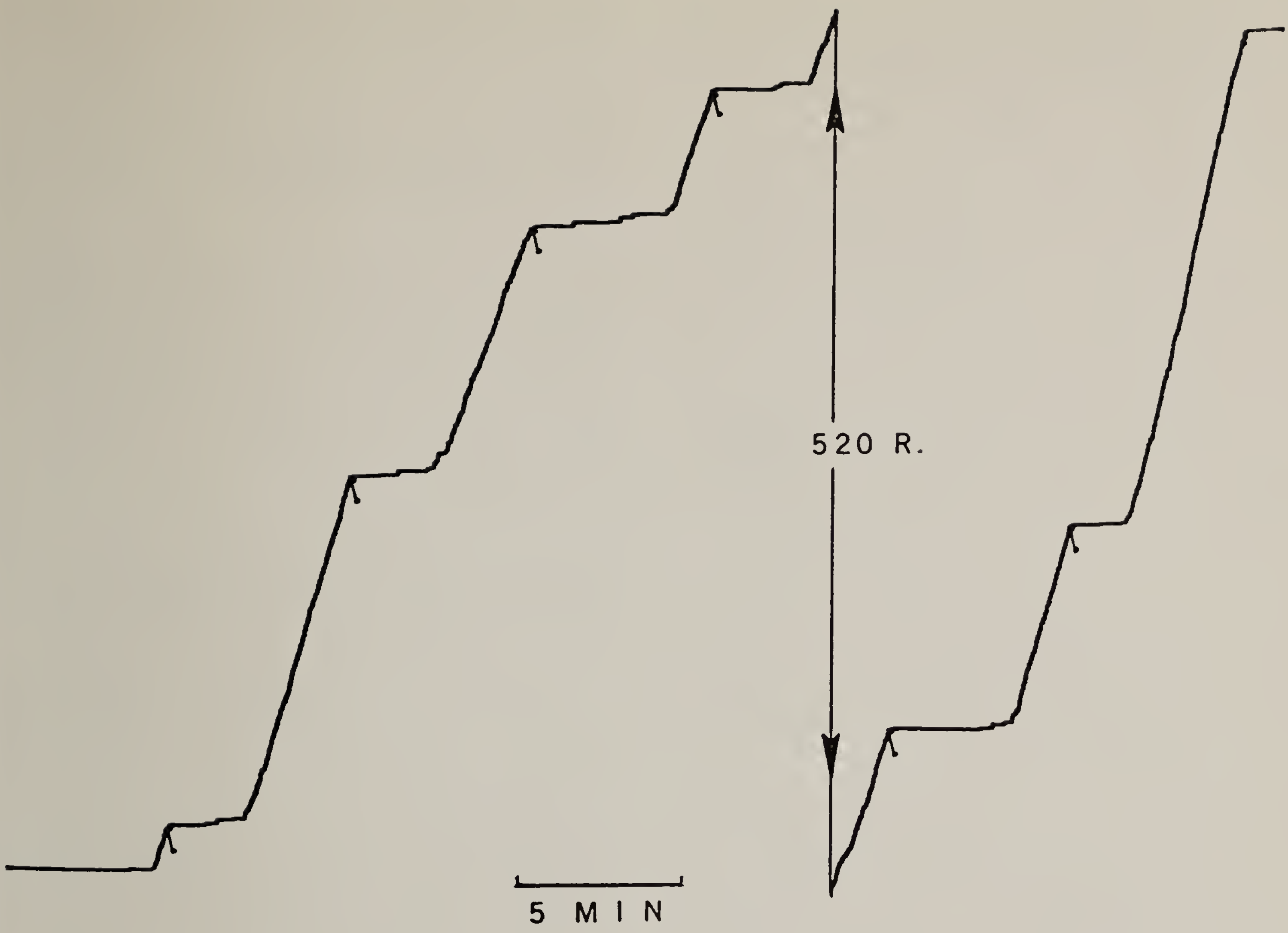

Fig. 3.—Bird 2. Sample of cumulative record of performance on Food key in Session 30 on the FI 5.5 schedule of reinforcement. Pips on the record indicate the occurrence of reinforcement. Very few observing responses occurred and no predictive stimuli were obtained. The performance shown was typical of that obtained at that stage of the experiment and also of that obtained on simple fixed-interval schedules.

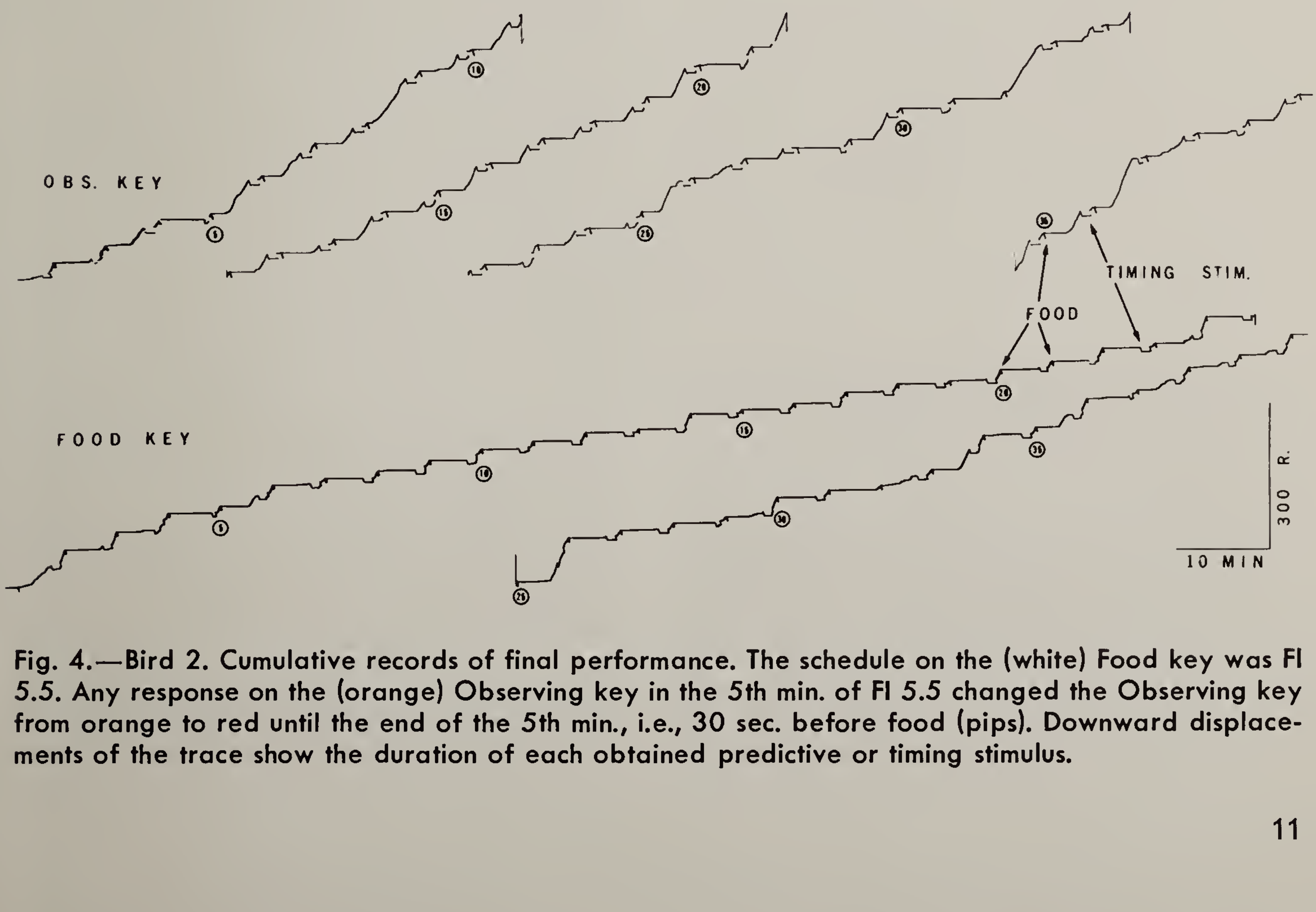

Fig. 4.—Bird 2. Cumulative records of final performance. The schedule on the (white) Food key was FI 5.5. Any response on the (orange) Observing key in the 5th min. of FI 5.5 changed the Observing key from orange to red until the end of the 5th min., i.e., 30 sec. before food (pips). Downward displacements of the trace show the duration of each obtained predictive or timing stimulus.

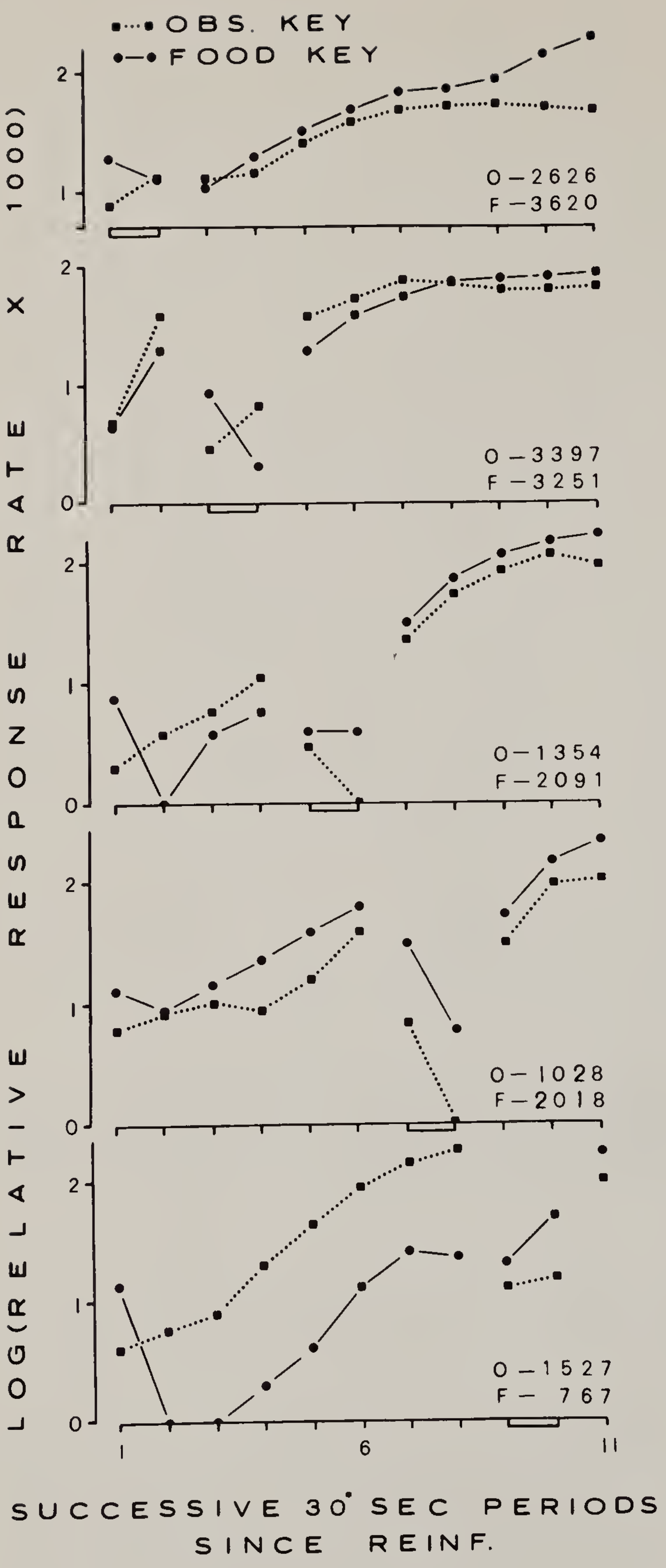

Fig. 5.—Bird 2. Relative response rate on both keys during successive 30-sec. periods. Each plot shows the final performance at a different value of T (T = 0, 1, 2, 3 or 4, reading from top down). Data points for the two periods in which the timing stimulus could be obtained are not joined to adjacent data points. Position of the timing stimulus is also indicated by the double line on the abscissa. Data from the last 30 intervals of a session contributed to each plot. The numbers of Observing (O) and Food (F) key responses emitted in the last 30 intervals of the session are shown at the right of each plot.

and 7, which show relative response rates on each key in successive 30-sec. periods with T = 0, 1, 2, 3 and 4. The data are from single representative sessions. The logarithmic ordinates have the effect of making differences between low relative rates larger (on a linear scale) than differences between high relative rates. The absolute response rates, in any 30-sec. period since reinforcement, can be calculated from the Figures, since the total numbers of responses are given. For example, in Fig. 5, at T = 0 the maximum log relative observing response rate is 1.5 or 0.015 of all responses. This corresponds to an absolute number of responses of .015 (2626+3620) = 94. These 94 responses were emitted in 30 X 30 sec. = 15 min. and therefore represent a rate of just over 6 responses per min.

Behavioral effects of the predictive stimulus as a function of T. Overall response rate measures of the effectiveness of the predictive stimulus as a function of T did not give uniform results for the three birds, though uniformity of results was improved in various derivative measures of response rate. Rather than give these derivative measures, we show, in Fig. 8, the proportion of available predictive stimuli actually obtained in the four final sessions at each value of T. The proportion of stimuli obtained varies directly with the value of T.

The reinforcing effect of the predictive stimulus is shown by the maintenance of observing behavior at all values of T. In addition, observing response rate accelerated in the fashion of FI performance up to the occurrence of the predictive stimulus; this ability of the predictive stimulus to exert schedule control is also evidence for its reinforcing effect.[8] The maintenance of observing behavior has dual significance in terms of traditional behavior theory. On the one hand it can be taken to show that a response can be strengthened by reinforcement delayed by several minutes. On the other hand, it can be taken to show that a stimulus may become a conditioned reinforcer when it precedes primary reinforcement by several minutes. However, we are inclined to doubt the significance of the distinction between strengthening a response and making a stimulus a conditioned reinforcer. We subsume both processes in our conclusion that the birds could detect a predictive relation between a stimulus and primary reinforcement and that the predictive stimulus reinforced operant behavior. Making the predictive stimulus contingent upon an observing response was a technique for measuring conditioned reinforcement of the predictive stimulus,

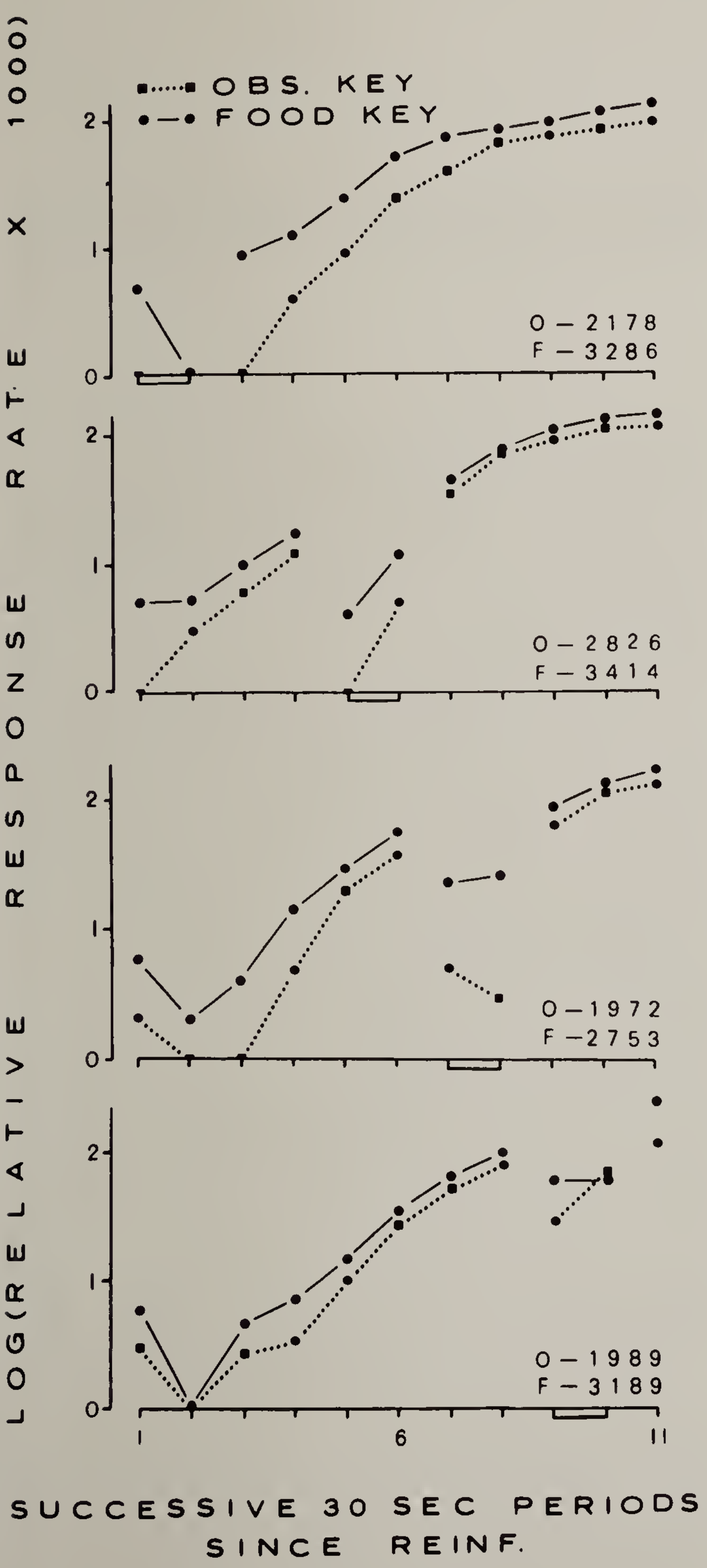

Fig. 6.—Bird 1. Relative response rates on both keys. See Fig. 5.The data at T = 1 were lost.

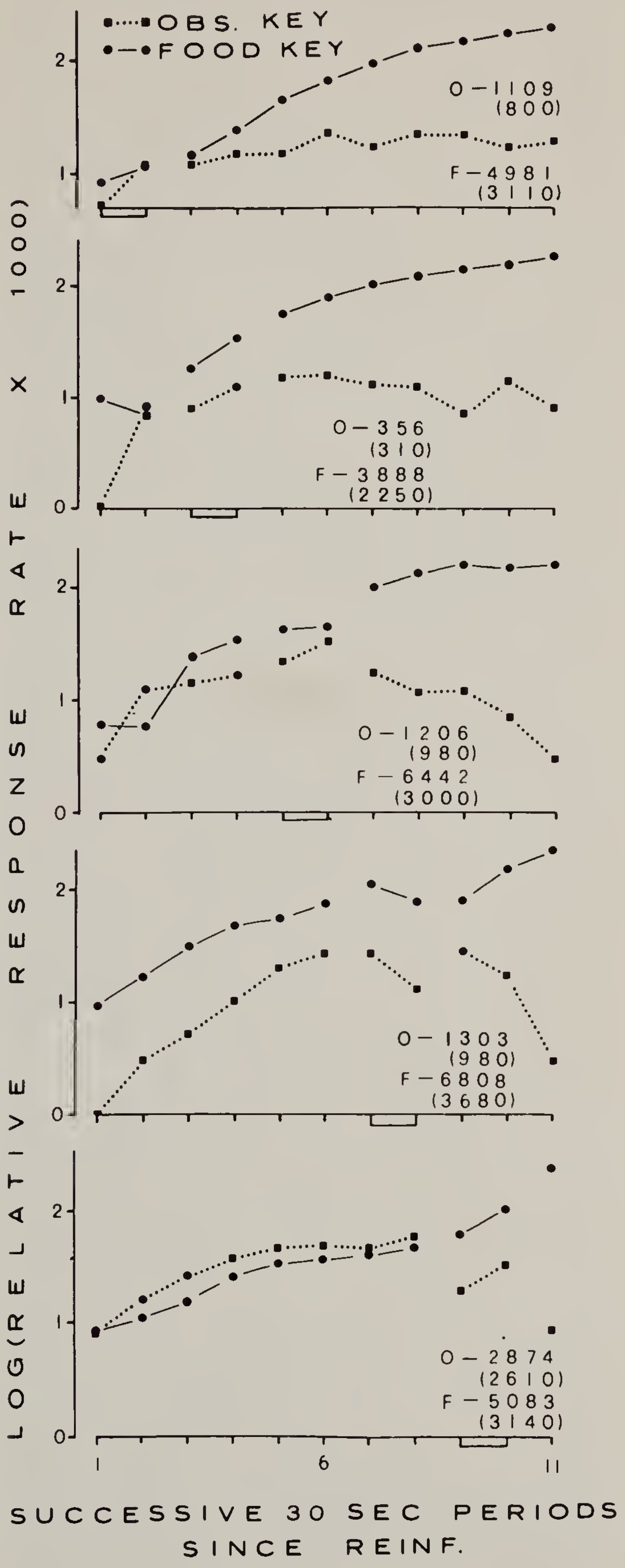

Fig. 7.—Bird 3. Relative response rates on both keys for a complete session. See Fig. 5. The numbers of responses on which the plots are based are indicated on the right of each plot. The numbers in brackets are the numbers of responses (measured from cumulative records) for the last 30 intervals, comparable to the numbers given in Figs. 5 and 6.

not a necessary arrangement for producing the conditioned reinforcing effect of the stimulus. In many circumstances, however, animals will fail to detect certain sequences of stimuli leading to reinforcement because of receptor orientation, complexity of the sequence, etc. Such failures no doubt account for the appearance of very limited behavioral capacity in many delayed-reinforcement experiments. Fortunately, it is a widely accepted principle of behavioral analysis that the onus of proof is on those who deny the existence of a behavioral capacity which has been experimentally demonstrated.

The predictive stimulus in this study was never closely associated in time with primary reinforcement. Even at T = 4 the stimulus terminated 30 sec. before primary reinforcement, a relatively remote association in terms of the time delays that usually are thought to attenuate effects of reinforcement. We must conclude that a predictive stimulus becomes a conditioned reinforcer when it predicts reinforcement by many minutes, up to an undetermined limit. In this study, even when the predictive stimulus terminated 4.5 min. before primary reinforcement, observing behavior was maintained and 30-40% of predictive stimuli were obtained (Fig. 8).

The general plan of the experiment incorporated all the usual safeguards against the involvement of mediating mechanisms of primary reinforcement. Thus, no exteroceptive stimuli differentially related to the occurrence of reinforcement occurred during the delay period, after the predictive stimulus terminated and before primary reinforcement was obtained. The birds could readily distinguish the two keys, as shown by the initial performance (Fig. 2) and the differences in later patterns of responding (Figs. 5, 6, 7). Also, there is no reason to suppose that response generalization from the reinforced response to the observing response via proprioceptive traces occurred, since the reinforced response was switched, now on the left key, now on the right. Moreover, one of the advantages of using the pigeon is that it is easy to bring a bird's behavior under the strong control of visual stimuli, which dominates the influences of stimuli in other modalities.

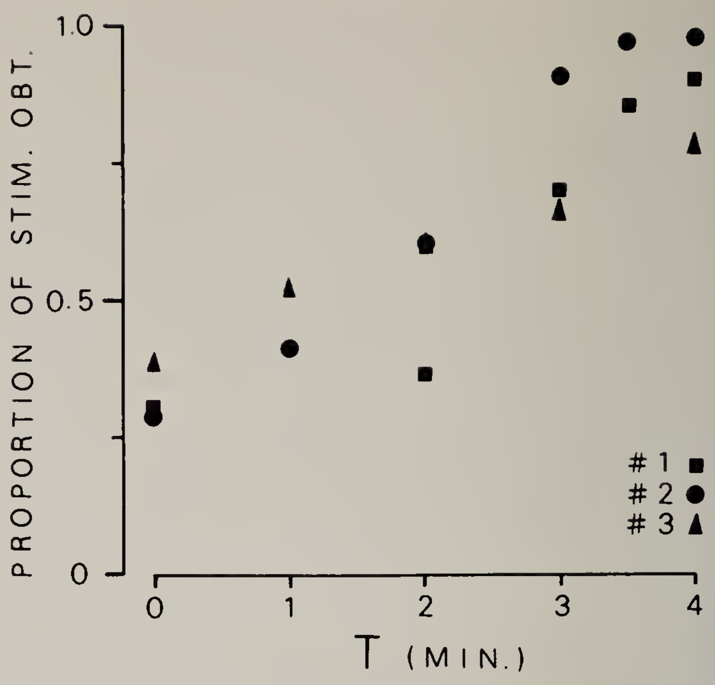

Fig. 8.—Proportion of available predictive stimuli actually obtained as a function of T.

There is, however, one way in which the delay period could have been "bridged." The end of the predictive stimulus signalled the start of a fixed-interval schedule of reinforcement on the food key. The results show typical fixed-interval behavior in this period, with positive acceleration of response rate. This may be seen in Fig. 4, but a representative cumulative record of performance in greater detail is shown in Fig. 9. Note the frequent occurrence of fixed-interval-like performance after the end of the predictive stimulus. If, then, the bird discriminates the short post-stimulus FI schedule, as it apparently does, this process effectively "stretches" the predictive stimulus up to primary reinforcement. The period after the stimulus is not empty, but is filled with systematic behavior. This could account also for the maintenance of performance with long delays of reinforcement in the study of Hendry and Dillow.[5] The fact that the predictive stimulus initiates a relatively short FI may also account for its reinforcing effect. Because the development of FI control is gradual,[7] one would expect the predictive stimulus, produced at first rarely and irregularly, to require many sessions to develop reinforcing value, as shown in Fig. 2.

The foregoing explanation suggests a prescription for making a conditioned reinforcer of a stimulus that predicts primary reinforcement by any length of time, namely, make the primary reinforcement contingent on a free operant, such as key-pecking or bar-pressing, so that FI performance will develop after the predictive stimulus. By contrast, the experimenter has no control over what the animal learns to do while confined in a delay box.

Relevance to second-order schedules. The present results appear to be relevant to those of Kelleher[9] with second-order schedules. In Kelleher's procedure, food was presented after completion of every *n*th fixed-interval schedule. Completion of the fixed-interval schedule was indicated by brief presentation of a key-light. An important detail is that the final key-light was presented just before the food. Kelleher's results showed that the key-light was a conditioned reinforcer and maintained fixed-interval patterns of responding. He concluded that the conditioned reinforcing effect is a consequence of the final temporal contiguity of the key-light and food presentation. The present results emphatically show that a stimulus can become a strong, conditioned reinforcer without being tempo-

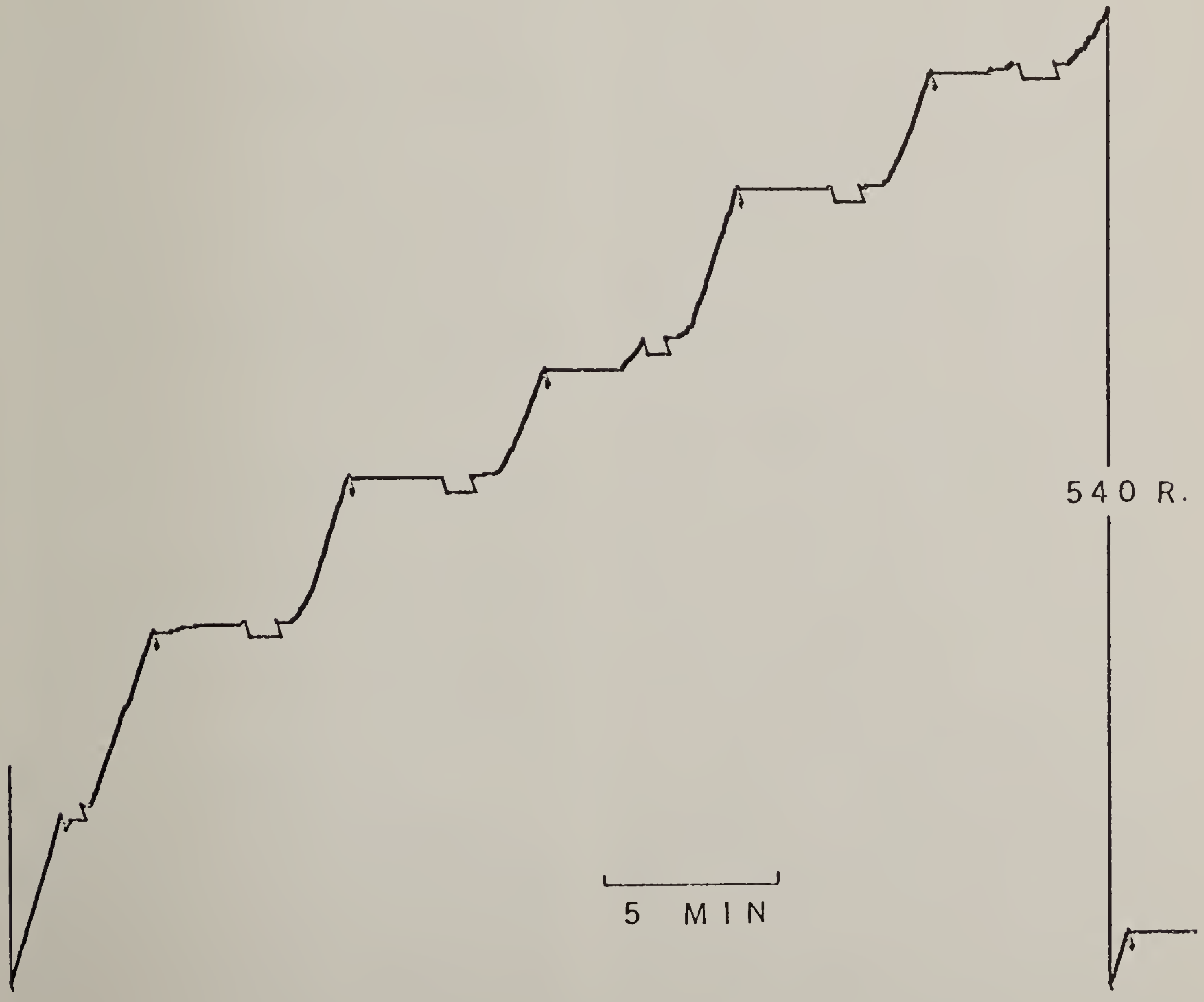

Fig. 9.—Bird 2. Representative sample of cumulative record of Food key performance at T = 2.5. The pips indicate reinforcement, and downward displacements of the trace show the duration of each obtained predictive stimulus.

rally contiguous with a primary reinforcer. Our results therefore suggest that typical second-order effects would be obtained by Kelleher's procedure with with the use of any distinctive stimulus change at the end of each component, rather than with only the stimulus that finally precedes food presentation. The stimulus becomes reinforcing by virtue of its predictive function whether or not it is also a conditioned reinforcer by virtue of its ultimate close temporal or discriminative relation to the response reinforced by food.

Dews has investigated a wide range of parameters of predictive stimuli in fixed-interval schedules.[10,11,12,13,14] The predictive stimulus in these cases is a change in illumination, even to complete darkness. In the simplest case, equivalent to the procedure of Farmer and Schoenfeld,[11] a single period of altered stimulus conditions is introduced during the fixed-interval. In more complex cases, several periods are used. Dews [12, 14] has drawn attention to the similarity between the procedure he has used and that referred to by Kelleher[8] as a second-order schedule. The description of the predictive stimulus in Dews' work as "S^{Δ}" suggests that the stimulus has aversive properties. The present analysis implies, on the contrary, that the predictive stimulus, or S^{Δ}, will be a positive reinforcer. In fact, Dews' procedure generates fixed-interval-like behavior up to each S^{Δ}, providing clear *prima facie* evidence for a positive reinforcing effect of the stimulus. There is a straight-forward, direct way to test whether the S^{Δ} in Dews' procedure is reinforcing: will the time-out itself reinforce observing behavior?

Theoretical considerations. It should be acknowledged that the view presented here of the function of the organism's perceptual and behavioral capacity to be selectively reinforced by cues that predict reinforcement is an implication of the comprehensive behavior theory of Tolman and Brunswik.[15] The theory is generally referred to as "expectancy theory" and was more or less displaced by S-R reinforcement theory. Expectancy theory, as developed by Tolman, had a strong flavor of rationalism, since he addressed himself to the general question: What behavioral functions must the organism possess if it is to have successful commerce with the environment? The shortest answer to this question might be: The organism must be sensitive to the "causal texture" of the environment. In other words, the organism's behavior must come under the influence of regular sequences of environmental events:

> "... the environment is a *causal texture* ... in which different events are regularly dependent upon each other. And because of the presence of such *causal couplings* . . . actually existing in their environments, organisms come to accept one event as a *local representative* . . . for another event. It is by the use of such acceptances or assertions of local representatives that organisms come to steer their ways through that complex network of events, stimuli and happenings, which surrounds them."[15]

It follows that:

> ". . . the organism has [the task] of developing an adequate reception system which will tend to select reliable cues, rather than ambiguous, non-significant or misleading ones."[15]

Perhaps this theory can be brought back to life by putting more empirical flesh on the rational skeleton. Much recent research seems to be pointing in this direction by showing that the organism is sensitive to and may behave on the basis of redundancies in sequences of stimuli.[16,17,18,19,20]

SUMMARY

This experiment was prompted by a re-examination of the procedures in delay-of-reinforcement experiments. Birds were reinforced on a 5.5 min. fixed-interval schedule. Observing responses on a second key produced a predictive stimulus which lasted at most 1 min. The position of the predictive stimulus relative to the reinforcement was systematically varied. In all positions the predictive stimulus was effective in sustaining observing behavior and controlling performance. The results show that the subjects could detect and act systematically on the basis of a predictive relation between a stimulus and primary reinforcement. The performance was not mediated by exteroceptive stimuli intervening between the stimulus and the reinforcement, but may have been mediated by systematic schedule-controlled performance in that period.

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PREOPERATIVE IRRADIATION: CRITICAL ANALYSIS: A REVIEW

William DeWolf

INTRODUCTION

Preoperative irradiation has been discussed and occasionally used for many years; reports on its effectiveness have varied from one extreme to the other· Pfahler[1] was first to use it with regularity. He noticed that with combined preoperative and postoperative irradiation, the five-year survival rates of patients with breast cancer increased significantly. In 1939, Myers[2] commented on the current therapeutic problems of his time, saying, "There are advocates of irradiation alone, advocates of preoperative irradiation, and a combination of preoperative and postoperative irradiation. With preoperative irradiation we are convinced there will be a noticeable improvement in the number of five year cures." In contrast, Adair[3] in a well-controlled study using 1810 cases of carcinoma of the breast came to the conclusion that preoperative irradiation not only does not improve five-year survival rates, but indeed may decrease them! In his words, "This experiment . . . largely sounded the death knell for preoperative irradiation." Mark[4] in his study of 60 patients with bronchogenic carcinoma said of preoperative irradiation that there are "higher operative mortality and postoperative cardiac complications with little difference in long-term survival rates."

Borgstrom[5] stated: "preoperative irradiation has no effect on survival," while Moss[6] believed that, "Whenever postoperative irradiation is necessary, preoperative irradiation should have been given."

Present-day opinions on the value of preoperative irradiation are not significantly more formed than those of the past. In order to form a reasonable opinion, one must first have a basic knowledge of the effects of radiation on tissues, both cancerous and normal. These effects then must be related to the dynamics of the neoplastic process and to the role of the surgeon.

ADVANTAGES OF PREOPERATIVE IRRADIATION

Better ability to destroy cancer cells: One of the purported aims of preoperative irradiation is not to sterilize the tumor but to "localize" it. This is accomplished by sclerosing vascular channels and destroying cancer cells at the tumor periphery. If operation precedes irradia-

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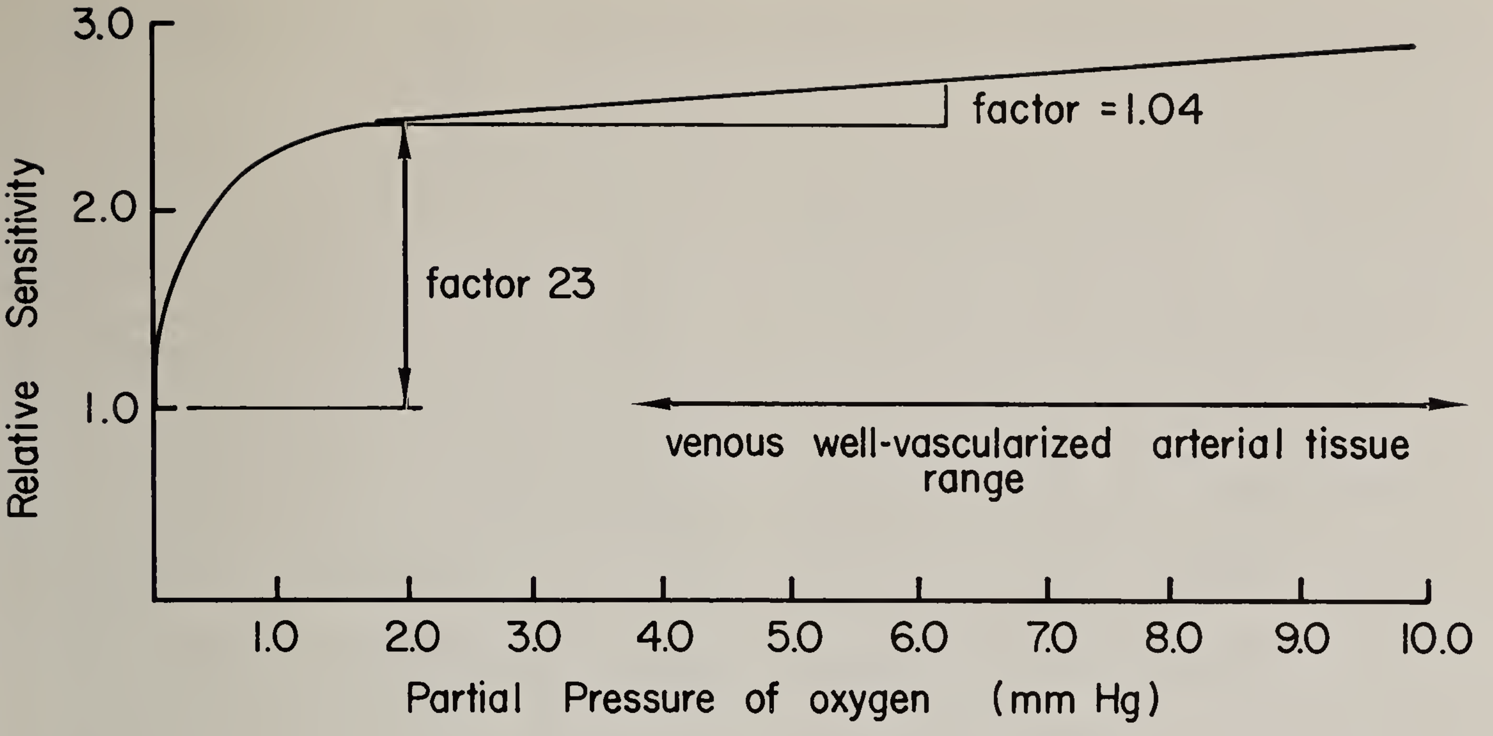

Fig. 1.—Cellular radiosensitivity relative to partial pressure of oxygen.

tion, the effect of radiotherapy is lessened, because the effect of surgery interrupts the integrity of the tumor and the tumor bed which decreases radiation response of the tumor.[7,8]

An oxygen effect, probably is responsible for the decreased radiosensitivity of tumors following surgery. This has been found in both *in vivo* and *in vitro* experiments. Aerobic cells are approximately 2.3 times more radiosensitive than anaerobic cells[9] (Fig. 1) such as those found in the fibrotic scar tissue following surgical trauma. Gray[10] has constructed a dose-survival curve which illustrates the effects of oxygenation on tumor radiosensitivity (Fig. 2). It shows that the number of cells surviving a dose of 4500r is 10^7 smaller when aerobic, rather than anaerobic, conditions exist. Also, by using a dose of 4500r, and if there is to be no more than 1:10 chance for any cell to survive, an aerobic tumor mass may be 10^{11} cells (50 gm) while an anaerobic mass may be only 10^4 cells (0.008 gm) in size.

The cause of the oxygen effect, largely unknown, was once believed due to the inactivation of respiratory and metabolic enzymes. Woolen[11] suggests however, that the relationship of tumor radiosensitivity to pO_2 relates to the old principle that radiation is an energy transfer involving ionization and decomposition of water producing chemical reactions and resultant cellular injury.[12] This sequence involves the formation of

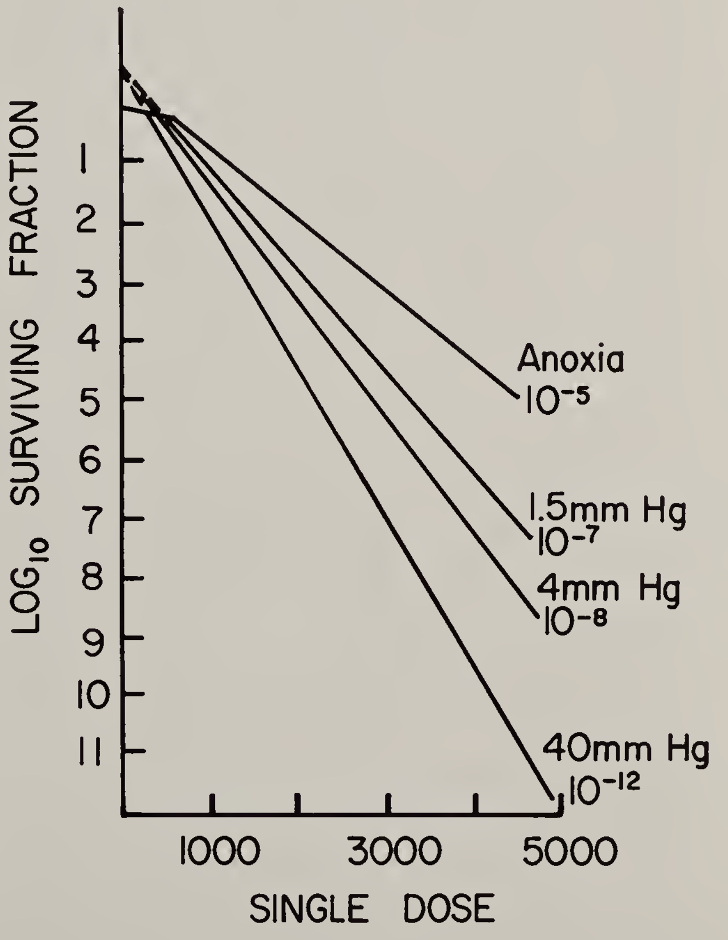

Fig. 2.—Survival of mouse tumor cells after a single dose of CO^{60} gamma rays at different oxygen tensions.

H_2O_2 which is an oxygen dependent reaction. Radiosensitivity, then, would be in part a function of the pO_2 of the tissue in question.

Devitalization of cells which may spread at the time of surgery: Tumor recurrence after an operation can be considered to result from at least one of three causes:[13,14]

a. dissemination at the time of operation as result of manipulation,
b. incomplete removal of tumor,
c. metastasis already present at time of surgery.

Preoperative irradiation of the intact tumor can prove to be of measurable advantage because a field larger than the primary surgical site can be irradiated, thereby not only controlling local extensions of the tumor but also affecting the surrounding tissue so that it will be more resistant to implantation should cancer cells spread at the time of surgery. This aspect of tumor therapy has been confirmed experimentally. Feder[15,16] administered various doses of irradiation to mouse rhabdomyosarcoma and then transferred the tumor via a curved surgical needle (which had been run through the tumor) to normal mice. He found that as the dose administered to the tumor increased from 1000r to 3500r, "takes" in the normal mice decreased from 100% to 50%, indicating decreased transplantability of irradiated tumor tissue. Vermund[17] and Summers[18] demonstrated that irradiation to a tumor bed rendered that tissue resistant to implantation. They irradiated the legs of mice with doses ranging from 1000r to 6000r, and 48 hours later injected these legs with spontaneous mammary carcinoma. Survivals for those receiving 1000r were 42% compared with 84% for those with 3000r, while the control mice which had not received radiation had a survival rate between 12% and 21%. They suggest several possible reasons for success in rendering a tumor bed unfavorable for growth, among them being: (1) connective tissue change, such as increase in collagen, inflammatory fibrosis, and compression and occlusion of capillaries; (2) decreased capillary formation within the irradiated area; and (3) changes in vascular function creating a barrier for nutrients. All of these processes serve to diminish the metabolic supply to the tumor. Inch and McCredie[19] demonstrated the ability of preoperative irradiation to decrease postoperative local recurrence by irradiating rats having Walker 256 Carcinosarcoma and mammary carcinoma implants with 2000r 24 hours prior to surgical removal. They found that the recurrence rate was significantly decreased as compared with rats receiving radiation 24 hours *after* surgery or having surgery alone.

Clinical evidence of the ability of preoperative irradiation to decrease the rates of recurrence is supplied by Henschke[20] who studied recurrences in 275 patients undergoing radical neck dissections for epidermoid carcinoma (Table 1). Preoperatively 111 patients received 2000r in five days. He demonstrated a 13% decrease in local recurrence in these patients. White, Fletcher, and Clark[21] observed similar results in 276 patients with cancer of the breast (Table 2). They found that of 68 patients receiving preoperative irradiation (4000r to axilla, 3000r to mammary glands in four weeks, with surgery six weeks later) there was a 10.4% decrease in recurrences at the end of five years.

The effect of preoperative irradiation on the behavior of tumor cells involves much more than control of local recurrence. According to Moore[22] "metastasis = number of cells in blood stream + biological activity + host resistance." Preoperative irradiation relates to each of these components in a very specific way. For example, the concept of biological activity has already been alluded to in the discussion of local recurrence and the ability of irradiation to decrease transplantability of tumor.

The notion that cancer cells are disseminated at surgery via manipulation and that the presence of these cells in the blood carries prognostic significance is still tenuous. The sequence of events in question can be represented as follows:

operative trauma and manipulation → dissemination of viable cancer cells → increased number metastases and decreased survival

Theoretically, preoperative irradiation should not only decrease the number of cells released by manipulation, but, in addition, reduce their viability. If there were no validity to this sequence, much of the advantage of using preoperative irradiation would be lost. Most authors agree that surgical manipulation does increase the chance of venous embolization.[16,23,24,25,26,27,] Roberts,[28] using cytological studies, demonstrated in six of seven cases a significant increase in circulating tumor cells after surgical manipulation.

Theoretically and experimentally this increase in circulating tumor cells takes on much importance. Zeidman[29] injected serial dilutions of Sarcoma 241 into the tail veins of mice, and 18 days later observed that the number of pulmonary metastases was directly proportional to the number of viable tumor cell "emboli" released into the bloodstream.

The clinical recognition of circulating tumor cells has been reported for 100 years beginning with Ashworth[30] who in 1869 noted cells in the bloodstream resembling those of multifocal skin tumor. Moore[31] found that 93 of 179 (52%) preoperative patients had "positive" blood samples while 60 of 109 (55%) of operative patients were positive, thus indicating no significant increase in circulating tumor cells during surgery. He later comments that "the additional release of tumor cells into the blood by surgical manipulation would not be more important than that caused by daily peristalsis and acute body movements."[31] Roberts[32] though, in a well-controlled study of 73 patients, found a 38% two-to-five-year survival rate in patients with negative blood samples for tumor cells during surgery as compared to a 20% two-to-five-year survival in patients with positive blood samples. Delarue[24] studied 270 patients and found a 33% two-to-five-year survival in those with positive blood samples, and a 68% survival in those with negative samples. Watne[33] in a study of 551 patients noted a significant decrease in 18-month survivals with the appearance of tumor cells in the blood. Table 3 shows the combined results of the various reports presented. Evaluating these figures is very difficult—one must consider timing of samples, amounts, place (peripheral vs. regional), and the method of collection as well as the identification of the desired cells. Indeed, the presence of tumor cells in the bloodstream may not be the principal factor

TABLE 1

Group	Number of Patients	% Histologically + nodes	% Local Recurrences
No X-ray	164	60	33
Preoperative X-ray	111	65	20

TABLE 2

Group	Number of Patients	% Local Recurrences
Preoperative X-ray	68	8.8
Radical mastectomy + Postoperative X-ray	208	19.2

TABLE 3

Authors	No. Pts.	% Survival Rates with . . .		Survival Time
		Positive Samples	Negative Samples	
Roberts[32]	73	20	38	2-5 years
Engell[83]	125	37	55	5-9 years
Delarue[24]	270	33	68	2-5 years

in the determination of metastasis, but appears to be highly significant.

In addition to manipulative trauma, it is thought that surgery may have a second role in promoting metastatic disease; it may reduce the resistance of the host so that hematogenous emboli are more likely to implant. Fisher and Fisher[52] noted that when they injected Walker 256 Sarcoma cells into rats, post-injection celiotomy increased hepatic metastasis by a factor of four. Studies on control animals showed that this increase was not due to the anesthesia, the dorsal incision, or intraperitoneal injection of air. Also noted in the experiment was that the chance of hepatic metastasis was the same regardless of when the celiotomy was done, perhaps indicating that the tumor lay dormant until some metabolic or stressful change favored its growth. Buinauskas[35] in a similar study found increased "takes" when Walker 256 Sarcoma was injected subcutaneously in rats after celiotomy as compared to controls which had no operation. Lewis[36] in an experiment controlled against dissemination from surgical manipulation also noted increased frequency of metastases and concluded the most likely explanation was the production of a decreased resistance to implantation. Guttman[37] returned this concept to the clinical realm by noting that in a series of patients with inoperable lung cancer, those treated by combined surgery and irradiation had a worse five-year survival rate than those treated by irradiation alone.

In theory, then, surgical manipulation appears to release malignant cells into the bloodstream. This increases the rate of metastases and local recurrence and decreases the survival rate. In addition, the stress of surgery decreases the ability of the host to resist metastatic emboli. Preoperative irradiation decreases the viability of tumor cells and protects the surgical site against implantation. This provides a measure of protection against local recurrence or peripheral dissemination and thus increases survival.

Conversion of inoperable lesion to operable lesion: The ability of irradiation to cause regression and localization of various tumors is well known. This phenomenon often renders nonresectable lesions resectable.[9, 38, 39, 40, 41] Bloedorn[9] mentions a series of 26 patients with preoperative irradiation, 13 of whom were originally considered inoperable, 12 on previous thoracotomy and one on clinical evaluation. All were eventually operated upon. Seymour,[42] likewise, in a study of 328 patients with carcinoma of the esophagus found that 56% treated by surgery alone had resectable lesions, while 84% treated with 4500r preoperatively were resectable.

The principle governing the use of preoperative irradiation in such cases is that local extensions of tumors, whether or not accessible to surgery, are more radiosensitive than the core of the tumor.[43] Preoperative irradiation of these extensions may render them nonviable through destruction of cells and sclerosis of lymphatics and the vascular bed. The total volume of the tumor tends to decrease and become better localized. The effect is to destroy the periphery of the tumor with irradiation and then, because of increased localization, allow a safer margin of resection.

THE DISADVANTAGES OF PREOPERATIVE IRRADIATION

Since the inception of preoperative irradiation, surgeons and patients alike have raised objections to its use. The following discussion will consider both sources.

Increased technical difficulty during surgery: When dealing with the effects of irradiation on tissue, one must consider several variables, such as dose given, time span of the entire therapy, size and number of treatment fields, alteration of blood supply, presence of infection, etc. Radiation during the early decades of this century tended to be unreliable because many tumor-doses were unknown. This alone may account for poor responses obtained. Present refinements in technology and utilization of fractionated supervoltage therapy have decreased radiation effect on normal tissue without sacrificing tumoricidal effect. Following the proper time-dose-surgery sequence (to be discussed later) surgeons report no increase in operative difficulty in cancer of the lung, breast, head, neck, and bladder.[2, 45, 46, 47, 48]

Increase in postoperative morbidity: In the early days of experimental preoperative irradiation, surgeons feared postoperative complications such as slough of tissue, fistula formation, and infection. Today, such problems have significantly decreased, the reasons being twofold: (1) There is a better selection of patients through improvement in diagnostic procedures and methods of determining operability. Many patients, seriously ill, are not subjected to "curative" surgery, with a resulting decrease in postoperative morbidity and mortality rates. (2) Better methods of irradiation are practiced. The use of supervoltage radiation with increased penetrability and skin-sparing effect, has done much to improve wound healing. It differs from orthovoltage (used previously) in its distribution of tissue effects and biological effectiveness. Orthovoltage radiation has its maximum ionization effect within the skin, while the shorter wavelength of supervoltage radiation results in maximum ionization and effectiveness three to four millimeters below the skin surface. Moist skin reactions are reduced or even eliminated. Improved wound-healing has been reported by many.[20, 21, 25, 42, 48, 49] White, Fletcher, and Clark[21] reporting on 346 patients with radical mastectomies, saw no difference in primary wound healing between those irradiated and those not irradiated before surgery (Table 4).

TABLE 4

Treatment	No. Pts.	% Primary Wound Healing
Radical mastectomy alone	196	80.1
Preoperative irradiation and radical mastectomy (Co^{60} 3000r in 4 weeks, surgery 5 weeks later)	150	81.3

TABLE 5

Treatment	No. Pts.	Hospital Stay	% Delayed Healing	Wound Slough
Non-irradiated	164	21 days	18	2
Preoperative Irradiation (Co^{60} 2000r 5 days)	111	21 days	25	2

Henschke[20] reported similar results in patients subjected to radical neck dissection for epidermoid carcinoma (Table 5).

In Henschke's series there is no difference in the length of hospitalization which is in accordance with the experience of Mark,[4] who found no increase in hospital stay in patients with bronchogenic carcinoma treated with preoperative irradiation. This is an important criterion in the evaluation of postoperative complications.

Cancer of the head and neck was one of the first to be treated with preoperative irradiation, and the procedure is widely used today. Numerous reports have since been published concerning the problems encountered with the use of preoperative irradiation.[48,49,50,51,52,53,54] Strong[49] reported on 111 patients who received 2000r of supervoltage x-ray prior to radical neck dissection for epidermoid carcinoma. His results definitely show no increase in postoperative complications (Table 6). Rupture of the carotid artery—probably the most serious of the complications following head and neck surgery—was studied in detail by VandenBerg[14] who found, in 294 patients operated under similar circumstances as Strong's, that the rate of hemorrhage in patients who develop fistulae or wound slough was higher in those with preoperative irradiation than in those treated by surgery alone. It is important to note, however, that the dosage of radiation was between 5000r and 6500r and the source was 250 Kv (orthovoltage) unit.

The most common complication of importance is fistula formation. According to the figures in Table 7, the surgeon operating in a previously irradiated field has no greater chance to encounter a postoperative fistula than the surgeon considering surgery as the single cure. When interpreting the significance of these numbers one must recognize that the tissue-effect of radiation is a measure of several important parameters, the limits of which are not all known. As seen in Table 7, patients receiving relatively smaller doses of radiation had decreased fistula formation. The dose including fractionation, the total duration of radiotherapy, the interval between irradiation and surgery, and the source of irradiation all play vital roles in determining the final condition of the operative field.

Whitmore,[55] in discussion of cystectomy for bladder cancer, presents a well-controlled study of 280 patients, of whom 50 received preoperative irradiation. He observes that, except for an increased pelvic wound morbidity, preoperative irradiation did not adversely affect the mortality or complication rates after cystectomy (Table 8).

TABLE 6

	Control (no X-ray)	Preoperative X-ray
Number of Patients	164	111
Complications (total)	59 (36%)	46 (41.4%)
Carotid Hemorrhage	1	2
Fistulae	(17%)	(10.8%)
Closed spontaneously	14	2
Closed surgically	14	10
Chylous fistula	3	2
Wound slough requiring 2° closure	2	2

TABLE 7

Series	No. Pts.	Treatment	Incidence of Fistula Formation	
			Preoperative X-rays	Control
Strong[48]	275	2000r	10.8%	17%
Vandenberg[14]	150	5000r-6500r	33%	32%
Ditchek[50]	19	6000r-7000r	10.5%	—
Powers[54]	30	1500r-3200r	3%	—
Silverstone[47]	21	5500r	43%	—
Morfit[53]	16	—	—	31%
Beahrs[84]	48	—	—	16%

Borgstrom,[5] in a comparable study of a series of 267 patients with stage I and stage II cancer of the breast (Table 9), shows that orthovoltage in the doses indicated does not cause an increase in wound complications. Note however that there is a significant increase in postoperative lymphedema in those patients treated with preoperative irradiation.

Delay of surgery: Patients often claim dissatisfaction with the one-to-three-month "waiting period" prior to surgery, during which preoperative irradiation is given. Many doctors have felt this represents psychological trauma to the patient. Others regard this length of time as necessary, because, while the primary tumor is being treated, occult metastasis already present at onset of therapy may become evident, thus changing treatment from curative to palliative and possibly eliminating the need for surgery.[56]

TABLE 8

Treatment	Postoperative Deaths	Complications	No complications
Radical Surgery: Cystectomy	32/230 (14%)	116/230 (51%)	82/230 (35%)
Preoperative X-ray + Cystectomy (4000r Co^{60} in 4-6 weeks)	8/50 (16%)	31/50 (62%)	11/50 (22%)

TABLE 9

Treatment	No. Pts.	% Wound Complications	% Lymph Edema
Radical Mastectomy + Postoperative X-ray (1400r)	122	40	43
Preoperative X-ray + Radical Mastectomy (8 days 1500r with 170 Kv)	147	35	63

Surgical biopsy needed prior to treatment: It is essential to confirm the presence of cancer by biopsy prior to instituting therapy. This, however, is not without some hazard. As previously mentioned when a tumor is surgically disturbed, the chances for dissemination of cells as well as local implantation are increased,[57,58] thus increasing the risk of metastases and local recurrence. Ackerman[57] believes that incisional or excisional biopsy, rather than needle biopsy, should be performed when possible because of the increased risk of tumor cells growing along the needle tract when needle biopsy is used. Kaae,[59] in a well-controlled study using 224 mice inoculated with mammary carcinoma, found that biopsy did not significantly increase the tendency for metastasis. Borgstrom[5] studied 269 women who received a radical mastectomy and postoperative radiotherapy for stage I or II breast cancer; in the 147 patients who received a small open biopsy prior to operation, biopsy did not affect survival rates.

Possible increase in frequency of metastasis: Clinically and experimentally, irradiation has often resulted in the opposite of the expected effect—that is, an increased number of metastases.[59, 60, 61, 62, 63, 64] Olch[61] found that 2.3% of mice given 3000r to implants of Cloudman S91 Melanoma had more than twice as many pulmonary metastases as control mice. Von Esson[64] utilizing mice with implanted mammary carcinoma, found that metastasis was three times as great if the tumor was irradiated with 800r as in those mice receiving no irradiation (Table 10).

Kaae[59] used 309 DBA mice injected with spontaneous mammary carcinoma. He divided them into three groups (Table 11). Group 1 was irradiated with 1000r, Group 2 with 2000r, and Group 3 served as controls. He found that the irradiated mice developed approximately four times as many metastases as the non-irradiated mice. Conclusions from this experiment were that if irradiation failed to control the tumor initially, metastasis might occur earlier than if no irradiation were given. In a study of 1,786 patients given 2000r of preoperative irradiation, Stearns[63] noted that liver metastases were more common than in control patients.

Many explanations have been offered to account for this effect of radiation on frequency of metastases. VonEssen[64] demonstrated that this effect was not due to

TABLE 10

X-ray Site	Inoculated Mice	Metastasis	
		No.	%
Tumor	33	15	45.5
Opposite Leg	28	3	10.7
None	26	4	15.4

TABLE 11

Group	No. Mice	Mice with Metastasis	
		No.	%
Control	92	5	5.4
1000r	82	21	25.6
2000r	135	30	22.2

mutagenesis but probably to an isolated effect on the tumor or tumor bed or to some unknown systemic effect. There are many host-tumor relationships as yet to be investigated; perhaps irradiation alters the cohesiveness of tumor cells, as first described by Coman;[65] perhaps there is an alteration of ameboid activity[66] of cancer cells or a disruption of the vascular system to provide an increased embolization effect. The answers are yet to be found.

EXPERIMENTAL RESULTS WITH PREOPERATIVE IRRADIATION

Many authors have applied concepts of preoperative irradiation to experimentation. Augustino[67] placed two-millimeter bits of Walker 256 Carcinosarcoma in the distal cecum-appendix of 200 rats. One hundred were irradiated with 200r, daily, for four days (250 Kv); 24 hours after the completion of irradiation they were operated upon. The results, as shown in Table 12, indicate that preoperative irradiation increased the resectability rate and the rate of survival, while decreasing the recurrence rates (in rats sacrificed at nine weeks). Powers,[54,68,69] in a classical study, also explored the effect of preoperative irradiation by subcutaneously implanting Gardner's Lymphosarcoma into each of the 200 mice. The mice were then divided into four groups (Table 13). Group 1 remained as a control, Group 2 received 500r (220 Kv), Group 3 received 500r (220 Kv) and surgery, while Group 4 received only surgery. The experiment was a single blind study which demonstrated the increased survival rate associated with preoperative irradiation. There was no observed difference in wound healing between Groups 3 and 4.

Each of these laboratory procedures exploits animal experimentation to its fullest. Clinical preoperative irradiation also involves detailed consideration of many more technicalities such as standardized observation time (five or ten years), dose-time fractionation, "wait-

TABLE 12

Group	No X-ray	Preoperative X-ray
A. Total number of animals	100	100
B. Number of animals resectable	79 ± 4.1	90 ± 3.2
C. Number of animals surviving 9 weeks	33 ± 4.7	49 ± 5.0
D. Number of animals showing no evidence of cancer at 9 weeks	21 ± 4.1	40 ± 4.9
E. Number of resected animals showing recurrence	58 ± 4.7 (73%)	48 ± 5.3 (53%)

TABLE 13

Group	1	2	3	4
Number of animals	50	50	50	50
Therapy	none	500r	500r + surg	surg
% 6 month survival*	0	0	85	53

*10 times needed for the slowest growing tumor to appear ing" period, as well as various surgical difficulties that occur. The experimental results do, however, encourage further investigation into the clinical use of preoperative irradiation.

CONCEPTS OF DOSAGE

For the maximal effective treatment of cancer with preoperative irradiation, it is important to consider the dosage, how it is given, and the time that elapses between irradiation and surgery. Oftentimes, when these variables are misjudged, survival rates fall far below those expected.

The main purpose of radiotherapy is to destroy cancer without damaging normal tissue. Older concepts[70] of radiosensitivity were based on the idea that neoplastic cells were more sensitive to the effects of radiation than normal cells. However, modern experimentation has shown that dose response relationships are similar for a wide range of mammalian cells, both normal and malignant, provided of course experimental variables (e.g., oxygenation) are the same. Thus, it is not always possible to administer the dose to which the tumor would respond best for fear of seriously injuring normal tissues or, if surgery is contemplated after irradiation, rendering the tumor inoperable due to poor surgical conditions (fibrosis, edema, etc.). The theoretical answer to this problem lies in fractionating the total dose, which merely means that the total dose is given in several increments within a predetermined period of time. Fractionation takes cognizance of the difference in growth behavior of malignant and normal cells—normal cells tending to repair themselves more quickly than malignant cells.[71] *If treatments are then timed so that each falls into the recovery period (of non-lethally damaged cells) of the tumor, but after the normal tissue has been completely repaired, then there will be a relative selective destruction of the tumor process.* The concept was first thought of by Regaud[72] who in his experiments with spermatogonia found that increasing fractionation tended to increase damage to spermatogonia while at the same time decreasing the damage to the scrotal skin.

The relationship between fractionation and the resultant biological effectiveness may be termed the "dose-effect" relationship. A typical graphic example of this is seen in Fig. 3.[72] The curve depicts a series of points representing the percentage of cells surviving after a given single dose of irradiation. The initial shoulder that occurs represents certain minimal unknown radiobiologic events which must occur in order for cell death to result. It is thus a measure of radioresistance and would vary with the radio-resistance of particular cell lines, as well as specific environmental factors which might be present that would alter the vulnerability of the cell (e.g., oxygenation). Thus, the more resistant the experimental object, the broader the initial shoulder of the curve; likewise, a sensitive cell line would have a sharp

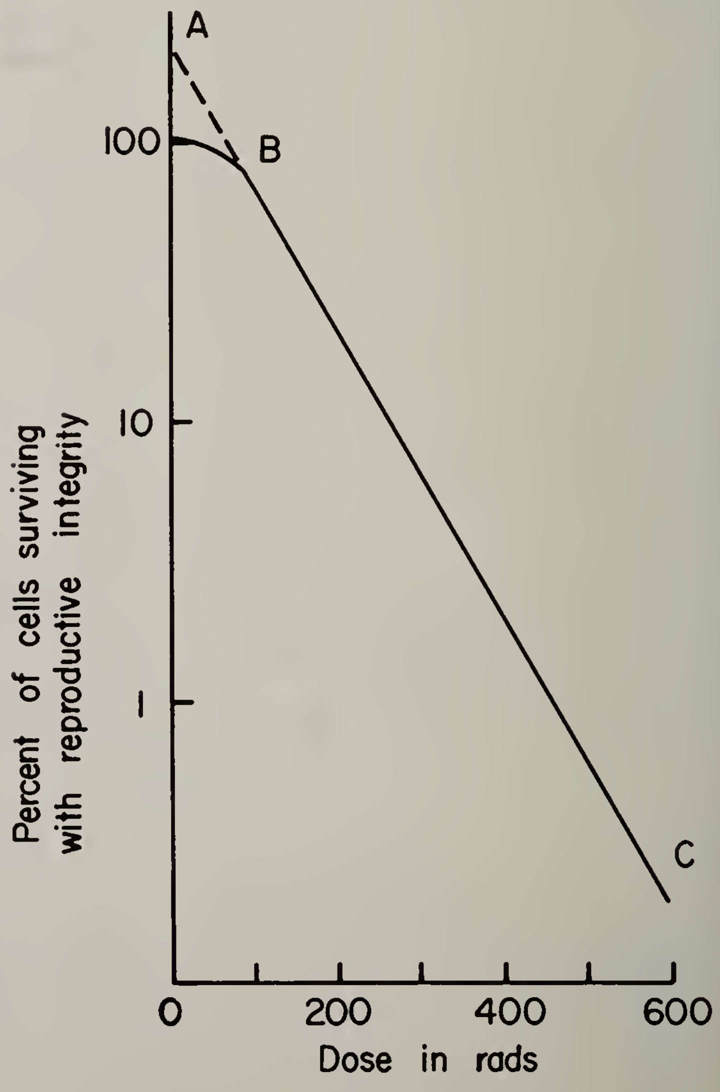

Fig. 3.—Cell survival curve.

curve. Similar to the shoulder of the curve, the slope of the straight part of the curve varies with the overall sensitivity of the cells: the steeper the slope the more sensitive the cells to irradiation. Elkind,[73,74] using cell lines from Chinese hamsters, showed that sublethally damaged cells could repair themselves to a pre-irradiation state, and that this repair was rapid, occurring within 24 hours. Hendrikson[75] found the same to be true; however, DuSault[76] has found the recovery period for skin to be 96 hours. Dosages must be increased along with fractionations in order to achieve the desired therapeutic effect. The advantage of fractionation, as mentioned previously, is that it tends to spare normal tissue, which tends to repair sooner than malignant tissue.

Experimental evidence regarding the repair of cells sublethally damaged by radiation and the subsequent effect of radiation on surviving fractions was demonstrated by Berry[77] who measured the "takes" of Ascites P-338 lymphocytic leukemia cells in mice after a prescribed amount of irradiation (Table 14). He found that the fractionation of radiation treatment does indeed decrease the lethal effect as compared with single dose administration, the effect being due to the increased chance of sublethally damaged cells repairing themselves.

Interposed between radiation therapy and surgery is the factor of time which plays an important part in determining the success of radiotherapy.[8] The surgeon and radiologist must consider:

1. Sufficient time for full action of radiation to take place;
2. Sufficient time for the tissues in the surgical field to be in best condition, thus striking a balance between acute radiation effects such as hemorrhage and edema and chronic effects such as endarteritis, fibrosis, and atrophy;
3. Insufficient time for those cells sublethally damaged to completely repair themselves; and
4. Practical reasons such as economics and the various hospitalization schedules imposed by these therapies.

TABLE 14

Treatment	Surviving Fraction of Cells (relative)
Control	1.0
1000r	0.1
2x500r	0.2
1500r	.026
3x500r	.089
2000r	.007
2x1000r	.017
4x500r	.028

In the past, this waiting period has varied from one day to one year. At present there is no absolute guide as to when to perform the operation; most of the information that can be relied upon has come from clinical experience. In the next section, various clinical results will be given.

CLINICAL TRIALS AND RESULTS

Preoperative irradiation may now be discussed in terms of its effect on survival as has been determined clinically. Variables which presents themselves are dosage and fractionation schedules, waiting period between x-ray and surgery, the radiation source (supervoltage vs. orthovoltage), the stage of cancer (e.g., nodal involvement), and the type of tumor to be cured.

One of the most inconstant variables seen is that of the "waiting period," that is, the interval of time between the completion of radiation and time of surgery. Clinically superior results are obtained when this period of time is less than one month, preferably between four days and two weeks. The most pronounced example of this is seen in the work of Nakayama.[41] His group of patients with carcinoma of the esophagus with nodal metastases with a waiting period of four to seven days, experienced an increase in three-year survival rate from 21.7% to 44.4%.

There are several technical reasons for proceeding with surgery soon after irradiation, the first being that a tumor will begin regrowth between two and four weeks after low-dose irradiation below cure attempt levels assuming, of course, the tumor is not 100% sterilized, as it usually is not. This was studied experimentally by Inch and McCredie,[78] who observed the effects of 2000r on subcutaneous implants of mammary carcinoma in mice. They noticed that after 10 days the tumors began to grow and increase in size. Halley[70] studied the effects of 1200-4500r on human breast carcinoma and found that tumor cells began to grow after four weeks. Nakayama[41] has shown that, with carcinoma of the esophagus, after 2000r there is a regression of the therapeutic effects of radiation after two weeks, which becomes pronounced at one month.

The second reason for early post-irradiation surgery relates to those findings of Stearns[79] in his study of rectal carcinoma. He noticed that as the time lag between irradiation and surgery increased, so did the incidence of metastases to the liver. This is associated with a relatively constant metastatic rate in those treated with surgery only, the implication here being that the longer the waiting period the greater the chance for metastases to occur.

A third reason pertains to the increased incidence of operative complications that occur with late operations. Vandenberg[14] studied this problem in detail by correlating the waiting time with the number of postoperative complications in 124 patients receiving 5000-6000r (four to six weeks 250 Kv) prior to radical neck dissections. Criteria for postoperative complications were the incidence of fistulae, wound sloughs, infections, and rupture of the carotid artery. The results indicated in Table 15 show that operations performed within the first month have less than half the chance of complications of those performed after two months. No patients operated upon in less than one month after radiotherapy had a wound slough. The success of surgery at this time was attributed to the fact that fibrosis and endarteritis are minimal, while tumor regression is maximal.

TABLE 15

Interval between radiotherapy & operation (months)	Patients	Complications	%
1	21	3	14%
2	30	6	20%
3	32	11	34%
4-6	33	11	33%
7-12	20	8	40%
12+	9	3	33%

Of equal importance is the amount of radiation given in the initial phase of treatment. Nickson[80] in his discussion of preoperative irradiation distinguishes doses as either "full" doses (greater than 3000r) or "moderate" doses (2000-3000r) and recognizes the "apparently favorable effect of moderate doses." Aside from the excellent results of Stearns[63] and Nakayama,[41] who have used relatively small amounts of radiation, there are other reasons for believing moderate doses to be favorable. Experimental as well as clinical results seem to indicate that approximately 3000r offers the maximal effect on the tumor with minimal effect on mortality (assuming, of course, that the tumor is sensitive to radiation). Nakayama,[41] in a study of the mortality rate of resection of irradiated carcinoma of the esophagus (Table 16) noted an enormous increase in deaths when the dose was increased over 3000r. Likewise, Kaae,[81] in a study of 492 cases of breast carcinoma, found that heavy preoperative irradiation afforded no better survival than moderate doses. Summers,[18] in an experiment utilizing mammary adenocarcinoma in mice, found that survival rate and growth-inhibition of tumor increased linearly with doses up to 3000r.

Doses higher than 3000r produced little effect on either of these two variables.

TABLE 16

Dose	Number of patients	Deaths	Rate %
Less than 2000r	49	1	2.0
2000r—3000r	70	1	1.4
Greater than 3000r	35	4	11.4

Andrews,[82] and Friedman,[83] by constructing iso-effect curves for both squamous cell carcinoma and breast carcinoma, show that maximal radiation effect can be achieved in both these tumors with doses below 3000r if the dosage schedule (fractionation) is correct.

The value of preoperative irradiation is far from being completely and accurately determined. It has been of significant value in a few experimental trials, and merits further investigation and use in the treatment of specific neoplasms.

SUMMARY

The status of preoperative irradiation has advanced relatively little since the days of its inception—the knowledge of its presence is perhaps more widely known, but its use is still looked upon with great skepticism and hesitancy. The reasons for this may be threefold: first, it actually seems quite logical to treat cancer first by removing it and then "cleaning up" the area with radiotherapy; second, preoperative irradiation has been tried in the past with only equivocal results; and third, the basic principles behind its use are not widely known.

This paper attempts to refresh the reader's mind regarding the use of preoperative irradiation by pointing out its advantages, disadvantages, and the reasons for each. The evidence suggests that it may be more logical to give radiation *before*, rather than *after*, surgery. It is pointed out that modern radiotherapy with the use of supervoltage radiation is far better than the orthovoltage radiation of days past—thus the equivocal results obtained from early experiments are not in accordance with present day findings.

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ABSTRACTS AND REVIEWS

OF PUBLICATION BY THE STAFF

Millburn, Lowell, Faber, L. Penfield, and Hendrickson, Frank R.: Curative Treatment of Epidermoid Carcinoma of the Esophagus. *Amer. J. Roentgen., 103:291-299, June, 1968.*

Supervoltage radiation equipment has allowed the radiotherapist to deliver adequate doses of ionizing radiation to structures deep within the body without significant damage to superficial tissues. Such organs as the esophagus have benefited by this new ability. Several centers throughout the world have reinstated radiation therapy as the primary treatment of esophageal carcinoma.

The results of radiation therapy in the upper half of the esophagus are consistently, but not dramatically, better than those obtained with surgery, but the morbidity, mortality, and the applicability of surgery to lesions at the arch of the aorta and above makes surgery unwarranted. The surgical literature presents data which prohibit curative surgery for epidermoid carcinoma of the esophagus in this location. Functionally, however, radiotherapeutic curative results are not always satisfactory due to stricture and narrowing of the esophageal lumen. Perhaps in selected patients who are sufficiently incapacitated, appropriate interposition procedures may afford significant benefit. One may argue that lesions below the arch of the aorta are amenable to surgery; however, in the western literature, surgery alone has had uniformally poor results in controlling squamous cell carcinoma of the esophagus even in this location. The results are no better than with radiation therapy alone and the morbidity and mortality are considerably greater. For lesions in this location in patients who are considered good surgical risks, a preoperative course of radiation therapy followed by total thoracic esophagectomy, adequate gastric resection and lymph node dissection, and a proper interposition procedure may offer better functional results than radiation therapy alone as well as improve survival figures. To substantiate this latter point, one must look to the future reports however, which will be coming from Parker and Gregorie, Clifton et al. and other centers which are participating in combination preoperative irradiation and surgical intervention for squamous cell carcinoma of the esophagus.

Millburn, Lowell, Hibbs, George C., and Hendrickson, Frank R.: Treatment of Spinal Cord Compression from Metastatic Carcinoma. *Cancer, 21:447-452, March, 1968.*

Nearly 100 articles on the treatment of spinal cord compression from malignant disease are found in the literature. The articles reviewed here represent the largest and the best-documented of those found in the literature. The extreme majority of reports are from the surgical literature yet nearly all successful cases had protracted courses of radiation therapy following their surgical procedures. The literature fails to stress the morbidity or mortality of surgical intervention on patients with metastatic carcinoma. The definitions of expediency and immediacy, in terms of spinal cord compression are re-evaluated. The concept of massive-dose rapid radiotherapeutic decompression of the spinal cord is presented. The value of postoperative radiation is substantiated by the surgical literature. An attempt is made to answer the question of "When is pre-irradiation surgery necessary?"

Millburn, Lowell and Hendrickson, Frank R.: Radical Neck Dissection: The Follow-Up. *Laryngoscope, 78:270-278, Feb., 1968.*

We are in the process of evaluating all malignancies to determine whether a rational follow-up schedule can be based upon recurrence interval and critical duration of follow-up in primarily uncontrolled cancers. For the reasons stated we now offer a rational schedule (Table IV) for post-therapeutic evaluation of the patient who has had a radical neck dissection.

The site of the primary lesion and the type of therapy did not influence the time of recurrence in the neck, but the findings of the pathologist in the neck specimen determine greatly what the adequate follow-up must be.

Troelstra, A.: Detection of Time-Varying Light Signals as Measured by the Pupillary Response. *J. Opt. Soc. Amer., 58:685-690, May, 1968.*

The apparent brightness with which a light stimulus is perceived is determined not only by the average luminance of the stimulus, but also by its temporal properties. For instance, if the luminance is modulated periodically below the flicker-fusion frequency, the observed brightness appears, in general, to be higher than the observed brightness of an equal-average constant luminance. The pupillary response of the human eye, which seems to be well correlated with observed brightness, has been analyzed quantitatively as a function of the modulation index and frequency for sinusoidal modulation. Under certain quite general assumptions, it is possible to predict the presence of a peripheral linear low-pass filter in the visual system. It is shown how the characteristics of this linear filter can be determined from the experimental data. The break frequency turns out to be around 9 cps and the high-frequency cutoff has a slope of 18 dB/octave.

Garron, David C. and Cheifetz, David I.: Stimulus Rotation and Visual Scanning in Ss with Copying Disability. *Percept. Motor Skills, 24:1015-1024, 1967.*

Rotation of squares increases copying difficulty for young children and brain-damaged adults. Explanations stress both perceptual and cognitive factors. Records of visual scanning were used as an index of attention to areas of the stimulus field. Perceptual explanations were translated into scanning terms. The degree to which scanning is consistent with expectations inferred from perceptual theories was examined. Scanning of squares, rotated and unrotated, was compared for 7 normals and 7 adults with impaired copying ability. Variables were attention to contour, enclosed form, and ground. Conclusions were that: (a) normals scan rotated and unrotated squares similarly; (b) impaired Ss' scanning of rotated squares is different from normal scanning and from their own scanning of unrotated squares; (c) the differences found do not follow theoretical expectation.

Zuber, B. L.: Eye Movement Dynamics in the Cat: The Final Motor Pathway. *Exp. Neurol., 20:255-260, February 1968.*

Stimulation of the oculomotor nerve in the anesthetized cat with pulse trains sinusoidally modulated in frequency causes ipsilateral sinusoidal eye movements. The frequency response of this part of the final output pathways of the oculomotor system (oculomotor nerve to eye position) appears to be a first-order lag with a time constant of 64 msec. There is a nonminimum phase element equivalent to a time delay of approximately 15 msec.

Zuber, B. L.: Sinusoidal Eye Movements from Brain Stem Stimulation in the Cat. *Vision Res., 8:1073-1079, 1968.*

Ipsilateral sinusoidal eye movements may be produced in the anesthetized cat by stimulating the third nerve within the brain stem. Stimulation has been of the form of trains of pulses with a sinusoidally modulated pulse frequency. Sinusoidal responses are observed over the modulation frequency range of 0.5 to 20 c/s. These responses are characterized by a threshold and are obliterated by neuromuscular blocking agents. The sinusoidal output together with the results of static calibration indicate that the third nerve-medical rectus-eyeball system may be treated as a linear system for stimulus pulse frequencies between 50 and 150 pulses per second. Within this range the sensitivity of the system is approximately 0.12 degrees of eye movement/pulse per second. The static calibration curve of eye position versus stimulus frequency has the same shape as previously published curves of developed tension versus stimulation frequency.

Elstein, Arthur S., and Van Pelt, John D.: Dimensions in the Perception of Psychiatric Patients by Hospital Staff. *J. Consult. Psychol., 30:213-218, 1966.*

Agreement among psychiatric staff and the dimensions underlying such agreement in the perception of psychiatric patients were investigated using 2 Q sorts, a Behavior (B) deck and an Interpersonal Characteristics (IC) deck. Eleven patients were each rated by from 5 to 8 judges. Mean global agreement (r) about patients ranged from .22 to .52 on the B deck, from .20 to .43 on the IC deck; there were significant differences ($p < .001$) with both sets of statements in amount of agreement about patients. Factor analysis of the matrices of Q correlations yielded two identifiable factors in each. In the B deck, they were interpreted as Manageability (B1) and Level of Psychopathology (B2); in the IC deck as Anxious Intropunitiveness (IC1) and Treatability (IC2). Coefficients of congruence between B1 and IC1 and between B2 and IC2 were .95 and −.81, respectively. Factors B1 and IC1 were discussed as comprising a psychiatric hospital variant of the favorability or evaluative dimension regularly found in studies of person perception.

Zuber, B. L., Semmlow, J. L., and Stark, L.: Frequency Characteristics of the Saccadic Eye Movement. *Biophys., J., 8:1288-1298, 1968.*

Using a piecewise linear approach, individual saccadic eye movements have been Fourier decomposed in an attempt to determine the effect of saccadic amplitude on frequency characteristics. The characteristics were plotted in the traditional Bode plot form, showing gain and phase as a function of frequency for various eye movement amplitudes. Up to about one octave beyond the −3 db gain frequency, the limiting system dynamics represented by the saccadic trajectory of a given amplitude may be considered linear and second order. The −3 db gain frequency was used as a measure of bandwidth, and the −90° phase crossover frequency was used as a measure of undamped natural frequency. These two quantities were used to calculate the damping factor. Both bandwidth and undamped natural frequency decrease with increasing saccadic eye movement amplitude. The damping factor shows no trend with amplitude and indicates approximate critical damping. When compared with the normal variation of characteristics for a given movement, the frequency characteristics of fixed amplitude saccades showed no generalized trends with changes in direction or DC operating level of movement.

Wu, H. M.; Lewis, J. P.; Sky-Peck, H. H.; and Trobaugh, F. E., Jr.: Biochemical Studies of Spleens of Heavily Irradiated Mice Transfused with Bone Marrow. *Radiat. Res., 33: 50-65, Jan. 1968.*

The patterns of recovery of hematopoietic tissue in spleens of heavily irradiated mice transfused with graded doses of marrow cells have been studied. The weights of the spleens, DNA synthesis in each spleen, and the splenic contents of RNA, DNA, lipid, and protein were determined. In general, the postirradiation period could be divided into (1) a period of depression, (2) a period of regeneration, and (3) a period of normalization.

In the period of depression, changes were similar in all animals, regardless of the dose of protective cells.

During the period of regeneration, in all animals that received protective doses of cells, the recovery of all properties occurred and the patterns of recovery were, in general, similar. The first splenic constituents to return to normal were the contents of RNA and protein, followed in sequence by weight, by lipid content, and finally by the content of DNA. In animals that received a minimal effective dose of protective cells, regeneration was vigorous and the amounts of all attributes studied exceeded normal values. In those mice that received the highest dose of cells, regeneration occurred steadily to normal values with little or no overshoot. The time required to reach a normal value was inversely related to the dose of cells injected, and thus the results of any biochemical study depend on the dose of protective cells as well as the time post-protection.

Evidence has been presented that the quantity of DNA synthesized may provide the best biochemical predictor of recovery from irradiation. The doses of cells that permitted synthesis of normal amounts of DNA were those that provided recovery from lethal irradiation.

We feel that the data reported here and those from other investigators provide evidence for the existence of normal-seeking mechanisms and that further studies of biochemical characteristics of hematopoietic tissue recovering from heavy irradiation following the injection of graded doses of hematopoietic cells may eventually permit these regulatory processes to be identified and measured.

Lewis, J. P.; O'Grady, L. F.; and Trobaugh, F. E., Jr.: Studies of Hematopoiesis in Murine Spleens: Significance of Hematopoietic Colonies Formed on the Surface Spleens. *Cell Tiss. Kin.*, 1: 101-109, 1968.

Colonies of hematopoietic tissue are formed in spleens of lethally irradiated mice by the injection of small numbers of hematopoietic cells. Some of these colonies appear as surface colonies, others can be identified only in serial sections of the spleen. The present studies have related the number and cellular composition of total hematopoietic colonies in the murine spleen to their visual recognition on the splenic surface. These studies demonstrate that only 50% of the total colonies in a spleen are recognized as surface colonies and that of these colonies on the surface, approximately 80% contain erythroid elements. At least four factors play important roles in the recognition of hematopoietic colonies as splenic surface colonies: (1) dose of repopulating cells or hematopoietic stem cells injected into the irradiated animal; (2) location of colonies within the spleen; (3) size of colonies; and (4) cellular content of the colonies. These studies demonstrate that surface colony formation reflects primarily erythropoiesis and not total hematopoiesis.

O'Grady, L. F.; Lewis, J. P.; Lange, R. D.; and Trobaugh, F. E., Jr.: Effect of Erythropoietin on Transplanted Hematopoietic Tissue. *Am. J. Physiol., 215: 176-182, 1968.*

Hematopoietic colonies produced in lethally irradiated mice by the injection of isogenic bone marrow were used to study the influence of erythropoietin on the growth and development of hematopoietic tissue. Groups of mice were exsanguinated on each of 10 days following lethal irradiation. Endogenous erythropoietin was not detectable in the plasma until after the 7th day postirradiation, several days after the hematopoietic graft had been established. In animals which had been irradiated with 750 R, and injected with 15,000 isogenic bone marrow cells, the amount of erythropoietin available to the hematopoietic graft was varied by hypertransfusion, administering exogenous erythropoietin, or administering erythropoietin antiserum. As plethora was increased there was a decrease in the size and number of erythroid colonies. With sufficient plethora erythroid colonies disappeared but there was no change in nonerythroid colonies. Exogenous erythropoietin stimulated the reformation of erythroid colonies, the number and size of which was dependent on the dose of erythropoietin administered. Antiserum to erythropoietin caused a decrease in the size and number of erythroid colonies.

Lewis, Jerry P.; Passovoy, Mitchell; Freeman, Mary; and Trobaugh, Frank E., Jr.: The Repopulating Potential and Differentiation Capacity of Hematopoietic Stem Cells from the Blood and Bone Marrow of Normal Mice. *J. Cell. Physiol., 71: 121, April 1968.*

Using the hematopoietic colony technique, we have investigated the repopulating potential of bone marrow cells and leukocytes of blood from normal mice and have demonstrated that the frequency of hematopoietic stem cells in bone marrow is 50 to 150 times that of stem cells in the circulating blood. The differentiation capacity of these stem cells has also been examined. Results of comparative studies of the serial sections of hematopoietic colonies formed from marrow and blood leukocytes indicate that the differentiation capacity of stem cells from marrow and blood is similar, and that at least 80% of these cells differentiate along a single cell line. Thus, peripheral blood stem cells can effect a complete hematopoietic graft, establishing in the host, donor red cells, granulocytes and platelets.

The possibility that blood leukocytes may serve as a potential source of stem cells for hematopoietic transplants has been considered. Although blood contains stem cells, their frequency is so low as to make it unlikely that they would become a useful source of precursor cells for transplantation purposes.

O'Grady, L. F.; Lewis, J. P.; and Trobaugh, F. E., Jr.: The Effect of Erythropoietin on Differentiated Erythroid Precursors. *J. Lab. Clin. Med., 71: 693-703, April 1968.*

While studying the early stages of hematopoiesis, we have gathered evidence which suggests that erythropoietin is required for the maturation of erythroid tissue but not for the differentiation of the hematopoietic stem cells. In the lethally irradiated mouse, transplanted stem cells seed in the spleen, differentiate, and mature into discrete erythroid, granulocytic or megakaryocytic colonies. After the fifth day posttransplantation, the stem cells renew their own number. During the first 10 days the cells of the erythroid colonies double in number every 12 to 14 hours. In plethoric mice, erythroid colonies are not formed, there is no change in the number of nonerythroid colonies, and stem cell renewal occurs at the same time as in nonplethoric animals. Exogenous erythropoietin stimulates the formation of erythroid colonies in plethoric mice. If the erythropoietin is given for the 4 days immediately preceding death, colonies are produced which are similar in size to those produced by 8 days of stimulation. If the colonies which were stimulated for only 4 days had started from a single stem cell, their cells would have had to double in number every 6 hours, a figure that is inconsistent with the measured growth rate of erythroid colonies. These colonies must have originated from a cluster of cells, not functioning stem cells, which differentiated into erythroid committed cells at the time of seeding, doubled several times in the absence of erythropoietin, but which required erythropoietin to complete their development into recognizable erythroid elements.

Mattenheimer, H. and Adams, E. C., Jr.: The Peroxidase-like Activity of the Hemoglobin Complex. *Z. Klin. Chem., 2: 69-78, March 1968.*

There is a conflict in the literature as to whether haptoglobin enhances or inhibits the peroxidase like activity of hemoglobin. This conflict arises from: (1) the effect of the concentration of H_2O_2 on the activity, (2) the use of different parameters to express the activity. In our own experience using *o*-tolidine as the donor, we found that, depending on the concentration of H_2O_2, activation or inhibition by haptoglobin was observed when the maximum color development was the parameter for the activity. With low concentrations of H_2O_2 activation and with higher concentrations of H_2O_2 inhibition was found. However, when the initial reaction rate was used to express the activity, there always was activation regardless of the peroxide concentration.

The sequence of events when H_2O_2 is added to $HgbO_2$ and $HgbO_2$: Hp and Methgb and Methgb: Hp was followed by observing changes in the spectra. The results from the change of the spectra together with the reaction rate of curves of the peroxidase reactions led us to the conclusion that the complex formed between hemoglobin and haptoglobin enhances the peroxidase like activity of hemoglobin and that it also increases the susceptibility to the destruction by H_2O_2.

Meinert, Curtis L. and Schwartz, Theodore B.: The Relationship of Treatment to Weight in a Randomized Study of Maturity-onset Diabetes. *Ann. N.Y. Acad. Sci., 148: 875-883, March 1968.*

Evidence is presented that diabetic patients treated with phenformin lose more weight early in the course of treatment than do patients treated with insulin, sulfonylureas, or placebo. The comparative degree of weight loss under phenformin therapy is small, however, and it is not known whether the weight loss will be sustained. Time regression curves are presented for trend analyses.

Kornel, Ludwig: Corticosteroids in Human Blood: III. Plasma Levels of Free and Conjugated 17-Hydroxycorticosteroids in Normal Subjects and Patients with Cushing's Syndrome before and after Administration of Corticotropin. *Acta Endocr., 60: 13-35, Jan. 1969.*

As an extension of our study on corticosteroid metabolism in essential hypertension, plasma levels of "free," "polar free," glucuronide and sulfate conjugated 17-hydroxycorticosteroids (17-OHCS), as well as "total conjugated" 17-OHCS, have been determined in 13 patients with Cushing's syndrome and 56 healthy people, before and after administration of corticotropin (ACTH). Levels of these steroid fractions were also compared in normal subjects during rest and after intensive work. Furthermore, kinetics of the formation and disposal of each of these steroid fractions were investigated *in vivo* in 3 normal subjects and

3 patients with Cushing's syndrome, by means of radioisotopic tracer techniques. The results obtained indicated the following: (1) although *mean* levels of each of the estimated 17-OHCS fractions, both before and after ACTH, were in Cushing's syndrome statistically significantly higher than in normal subjects (exception: 17-OHCS-sulfates after ACTH), many *individual* values for steroid concentrations in Cushing patients were well below the upper limit of the normal range; (2) levels of conjugated and "polar free" 17-OHCS in these patients were much more often increased above the normal range than those of "free" steroids usually measured; moreover, levels of these steroids in the control specimens constituted a much better index of adrenocortical hyperactivity than those after ACTH administration; (3) arithmetical formulae were construed, taking into account both an absolute increase in steroid levels and a relative increase in levels of conjugated and "polar free" steroids above those of "free" 17-OHCS, which permitted a clear-cut separation of *all* patients with Cushing's syndrome from normal subjects; (4) in the sulfate conjugated 17-OHCS fraction in Cushing patients, the increase in levels of more polar steroids (extractable with ethyl acetate following solvolysis and removal of less polar steroids with dichloromethane) was statistically significantly greater than that of less polar steroids (extractable with dichloromethane), thus, patients with Cushing's syndrome resembled from this angle patients with essential hypertension; (5) when steroid levels in normal subjects were compared during rest and during intensive work, a proportional increase in all steroid fractions during work was noted; this points to a perfect homeostatic mechanism involving enzyme systems metabolizing cortisol under "normal" stressful conditions; (6) however, after ACTH administration, the increase in the levels of conjugated steroids in normal subjects considerably exceeded that of "free" 17-OHCS; in contrast, in Cushing patients the increase in the conjugated 17-OHCS after ACTH administration was relatively smaller than that of "free" 17-OHCS; this, it is believed, indicates that the level of activity of enzymes metabolizing cortisol is in patients with Cushing's syndrome closer to the upper limit of these enzymes' capacity than in normal subjects, possibly due to a chronic saturation with higher steroid concentrations; (7) the results of the radioisotopic tracer study of the kinetics of formation and disposal of various free and conjugated plasma corticosteroid fractions demonstrated that, under basal conditions: (a) biological half-life of cortisol in Cushing patients is shorter than in normal subjects, (b) rates of formation of conjugated and polar free metabolites are much faster in Cushing patients than in normal subjects; this indicates that patients with Cushing's syndrome develop adaptive mechanisms to metabolize chronically increased loads of cortisol, and explains why plasma levels of "polar free" and conjugated 17-OHCS are a better index of adrenocortical hyperactivity than those of the "free" steroids, usually measured.

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Protein Synthesis, Platelet Populations, and Platelet Functions

Congenital Anomalies of the Coronary Arteries: Review

Abstracts

THE PRESBYTERIAN-ST. LUKE'S HOSPITAL MEDICAL BULLE

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BILE: WIDE GAPS IN USEFUL KNOWLEDGE

W. G. M. Hardison

Bile formation serves two main functions in man: it is an excretory route for bilirubin and other substances metabolized by the liver, and it provides bicarbonate and bile salt to the small bowel to aid in fat digestion. In all mammals except man bile serves these functions without complication; in man, however, bile tends to form stones. It has recently been estimated that fifteen million persons in the United States currently have gallstones[1] and this number will probably increase as the mean age of the population increases. Unfortunately, knowledge of biliary physiology is greatest in those areas concerned with normal biliary function, and least in the area of gallstone formation, which causes the most trouble. Knowledge of hepatic excretory function, bile salt formation and metabolism, and the regulation of bile flow has certainly been useful in explaining a variety of diseases, but such diseases are relatively uncommon as compared to cholelithiasis or choledocholithiasis. Stone formation, however, cannot be considered as a phenomenon entirely separate from other aspects of biliary physiology—it is known, for instance, that bile salts are important for cholesterol solubility; also, the amount of fluid and electrolyte added to bile directly alters the concentrations and solubilities of various components. Therefore one should approach the problem of gallstone formation with a knowledge of general biliary physiology.

Regulation of bile flow:

Bile flow can be divided into three compartments depending on the stimulus: 1. bile salt fraction; 2. secretin fraction; 3. basal fraction.

Historically, the first fraction to be defined was the bile salt fraction. For many years, investigators have known that bile salts are potent choleretics. The reason for this was proposed by Sperber[2] in 1963. Bile salts are concentrated and excreted by the hepatocyte into the bile canaliculus (Fig. 1). Bile salts, which cannot diffuse out of the canaliculus, create an osmotic gradient which draws fluid into the canaliculus. This mechanism of bile production is analogous to the mecha-

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nism of urine production by osmotic diuretics. Any substance which is concentrated by the hepatocyte and excreted in the canaliculus is capable of producing an "osmotic choleresis"—examples include the dyes fluoresceine and phenol red.

A second potent choleretic in man is the intestinal hormone secretin. This substance, however, does not work at the canalicular level and does not operate by creation of an osmotic gradient; it directly stimulates the secretion of electrolyte (predominantly bicarbonate) and water, probably by the ductular epithelial cells. The composition of the fluid fraction stimulated by secretin is constant; only the volume of this fraction varies with the dose of secretin.[3] A discrete secretin fraction of bile flow is demonstrable in most species (guinea pig, dog, pig, man) but is notably absent in some (rat, rabbit). The teleologic significance, if any, of this fact is unknown.

Originally it was thought that without bile salt or secretin stimulation, bile flow would cease. Recently, however, investigators have decided that there is an irreducible basal bile flow not dependent upon secretin or bile salts. Although information is scanty, this fraction may result from the transport of sodium (with its osmotically obligated water) into the canaliculus by the hepatocyte.[4] Although investigators have not speculated on the physiologic significance of this fraction, they probably are comforted to know that when bile salts are low and secretin absent, the biliary tree will not wither into a system of desiccated, crusted tubes.

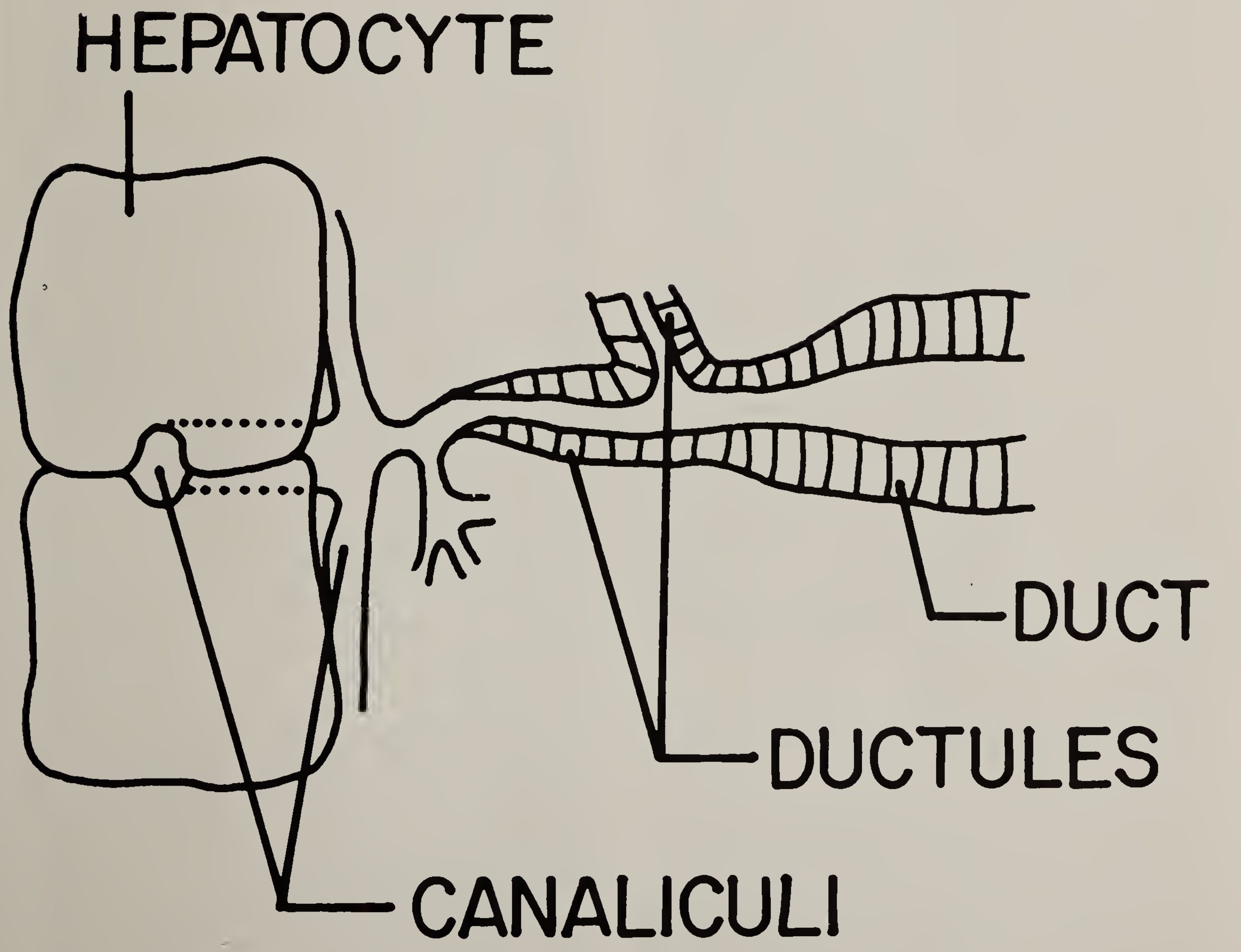

Fig. 1

Excretory function:

Ordinarily, in man, bile flow is composed of all three components or fractions. Since the first two fractions contain ample bile salt and bicarbonate, the function of supplying the small bowel with these substances is satisfied. Bile serves a second important function as well; it is an excretory route for a number of substances metabolized and excreted by the liver. Bilirubin is the most familiar of these substances. In addition, many of the hormonal metabolites produced in the liver are excreted in the bile: reduced conjugated steroid metabolities, thyroxine and throxine metabolites, and estrogen metabolites are a few. Finally, a large number of organic compounds metabolized by the liver are excreted in the bile; familiar examples are biliary contrast media, bromsulphalein (BSP), sulfonamides, erythromycin, some synthetic penicillins, and certain prophyrins. Most of these substances do not simply passively diffuse into bile; they are concentrated in bile to many times their plasma concentrations. This implies a specific active transport site or sites somewhere in the hepatocyte, most likely at the canalicular membrane. The presence of a specific transport mechanism shared by certain organic anions is suggested by the demonstration of competitive inhibition of transport exhibited by certain compounds (e.g. various bile salts by one another, taurocholate by phenol red). The presence of more than one transport mechanism, however, is dramatically demonstrated by the ability of Corriedale sheep, with a genetic defect in bilirubin and BSP transport, to transport bile salt normally.[5]

The gallbladder:

Thus far I have avoided mentioning the gallbladder. It is an organ which does more harm than good. Theoretically the gallbladder is a good idea. It receives hepatic bile and, through isotonic sodium chloride resorption, it concentrates bile 5 to 7 times. Bile salts so concentrated are then discharged under the influence of cholecystokinin into the duodenum where they are immediately available for fat absorption. However, this process probably represents wasted effort on the part of the gallbladder. Patients without gallbladders get along very well; and rats, who do not have gallbladders, similarly prosper. It is likely, in fact, that without the intervention of physicians and surgeons the gallbladder would have gradually faded from the human race by natural selection. Since the gallbladder will be with us for some time to come, however, the problem of gallbladder disease must be faced. Prevention of gallstones would be the best way to prevent most gallbladder disease.

Gallstones:

Research on gallstones and their formation has produced much speculation, a few hard facts and even less useful knowledge. Older theories which held that gallstones were caused by infection in the biliary tree, although probably correct in a minority of cases, have largely been abandoned. Rather it is believed that gallstones predispose to infection perhaps by the production of ischemia.[6] At present the cause of gallstones is unknown. However, several facts have been established which give direction to the search for an etiology.

With the exception of pigment stones seen in hemolytic states, gallstones are predominantly cholesterol. The reason that man is so susceptible to cholelithiasis is that his bile is, more than that of any other mammal, nearly saturated with cholesterol. Other animals handle biliary cholesterol well with a wide margin of solubility. This is shown by the rapid dissolution of human gallstones when placed in a dog's gallbladder. Man lives on a gallstone precipice; a small aberration in one direction may precipitate cholelithiasis. Admirand and Small have shown that gallbladder bile from patients with cholelithiasis is always supersaturated with cholesterol, whereas that from normals is not.[7] For specifics on the diverse theories of gallstone formation the reader is referred to a recent review.[8]

Gallbladder bile could become supersaturated with cholesterol at two sites: the liver cell might secrete bile abnormally high in cholesterol, or the gallbladder might function abnormally and produce supersaturation in a number of ways. Simple concentration of bile by the gallbladder is not the answer. Physicochemically such concentration actually increases cholesterol solubility because of concomitant concentration of other substances. Several observations suggest, but do not prove, that the fault lies in the hepatocyte rather than in the gallbladder. Patients with a history of cholecystectomy for stones may have cholesterol crystals in hepatic bile and may even form ductular stones. Patients with congenital absence of the gallbladder have been reported with common duct stones. Analysis of T-tube bile from patients has shown, in the hands of some investigators, statistically higher cholesterol outputs from patients with a history of stones than from patients without such a history. These findings all suggest that an abnormality may exist even in the absence of the gallbladder.

To understand what might cause abnormal bile, it is necessary to understand what factors control biliary cholesterol output and what factors control biliary phospholipid output. Cholesterol is totally water-insoluble. It can dissolve only in the presence of phospholipid and bile salt which form a molecular aggregate. This aggregate is capable of dissolving cholesterol in its center. To date, practically nothing is known in the experimental animal, let alone man, about factors influencing lipid (i.e., cholesterol and phospholipid) output in hepatic bile. Only the effect of serum cholesterol has been studied: serum cholesterol concentration does not correlate with biliary cholesterol concentration either in the experimental animal or in man. The effect of diet is only beginning to be investigated.

In recent studies using the rat, we have found that biliary bile salt concentration is closely related to biliary lipid excretion. Bile salt, by forming molecular aggregates with phospholipid, probably dissolves cholesterol as it passes through the hepatocyte. Although there is suggestive evidence that this also occurs in man it has not been definitely established. If true in man, this would mean that a "normal" biliary cholesterol concentration of a bile sample could be defined only with reference to the bile salt concentration, simultaneously determined, in the same sample. It is not enough to determine, for instance, that patients with cholelithiasis have higher biliary cholesterol concentrations than normal controls. If the patients have higher biliary bile salt concentrations, their biliary cholesterol concentrations might be normal and their bile be no more prone to cholesterol supersaturation than control bile. This fact may undermine many of the "comparison" studies done in the past. Likewise factors which cause small elevations in biliary cholesterol, which may well be the important factors in human gallstone formation, can be appreciated only by considering the level of cholesterol with reference to the coexisting bile salt concentration.

Much of the above is speculation and requires experimental demonstration. Such demonstration may be sought in two directions. Having established a normal biliary bile salt-lipid relation in the rat, we may now go on to examine under controlled experimental conditions the effect upon this relation of factors thought to promote gallstone formation: e.g. lithogenic diets or estrogen administration. Studies in man will necessarily be more difficult and less well controlled. Studies of biliary lipid concentration in T-tube bile as bile salt concentration first falls and then rises after cholecystectomy should be helpful. In time, the opportunity to study a patient with a chronic total biliary fistula may arise. To extend the range of bile salt concentrations in T-tube bile, we can infuse bile salt into the ileum. The ileum is equipped with an efficient active transport system for bile salts, and such a route would substitute in man for intravenous infusion of bile salt which can be done only in animals.

Whether these approaches will yield information concerning prevention of gallstones (i.e. useful information) remains to be seen. In the past several years, investigators have learned much about the physicochemistry of bile. This new knowledge has re-kindled interest in an old subject, and a number of groups are working on the problem of gallstone formation using a variety of approaches. We can hope that one or several of these diverse approaches will eventually allow prevention of gallstones and allow the gallbladder to do more good than harm.

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PROTEIN SYNTHESIS, PLATELET POPULATIONS, AND PLATELET FUNCTIONS

F. M. Booyse
K. W. Gilchrist
D. C. Hohn
T. P. Hoveke
D. Zschocke
M. E. Rafelson, Jr.

> "*Fie on thee, oh cursed platelet!*"
> *K. W. Gilchrist, 1969*

Proteins comprise some 55 to 60% of the dry weight of the human platelet. At the present, relatively little quantitative data are available for the individual proteins, and even less is known about their possible roles in platelet functions. The proteins so far identified in the human platelet are listed in Table 1. In speculating on the origin of platelet proteins, we considered it likely that the albumin, fibrinogen, and globulins probably originated from plasma, although we could not detect other plasma proteins such as transferrin, haptoglobin, oroscomucoid, ceruloplasmin, etc.[1] We also thought it possible that certain proteins might be synthesized by the platelet despite the fact that they are anuclear fragments of the megakaryocyte cytoplasm and contain no nuclear DNA. To our knowledge, protein synthesis had never been investigated in the platelet. Since the first step in the informational flow required for protein synthesis was absent, the transcription of DNA coding to messenger RNA, it was probably presumed that the subsequent steps would not be operative.

In a series of experiments we demonstrated that protein synthesis did occur in the platelet and that a stable messenger RNA directed the *de novo* synthesis of

TABLE 1. PROTEINS OF HUMAN PLATELETS

Constituent	% of Total Protein
Contractile protein (thrombosthenin)	15–20
Fibrinogen	15–20
Albumin	2–3
Glycogen-associated globulin	5–6
Prealbumin	?
Plasminogen	?
γ-Globulin	?
γ_1-Macroglobulin	?

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the actomyosin-like contractile protein.[2,3] Other laboratories subsequently also showed protein synthesis in platelets.[4,5,6] The *sine qua non* to demonstrate conclusively the ability of platelets to carry out protein synthesis was the preparation of a cell-free system which would carry out this process. An active, reproducible cell-free system from platelets was obtained which has the characteristics shown in Table 2.[7] As may be seen, the system appears to be a conventional one in that amino acid incorporation is dependent on the presence of ribosomes, a soluble enzyme fraction, ATP, and an ATP-generating system. Both puromycin and ribonuclease inhibited amino acid incorporation.

TABLE 2. CHARACTERISTICS OF AMINO ACID INCORPORATION IN CELL-FREE SYSTEM FROM HUMAN PLATELETS

Incubation Medium	Counts/min
Complete system*	3600
Minus ribosomes	50
Minus soluble enzymes	200
Minus ATP, GTP, and ATP-generating system	350
Plus Puromycin (150μg)	120
Plus Ribonuclease (15μg)	450

*The complete system contained in 1 ml, 50μg ribosomes, 2 mg soluble enzymes, KCl in a final concentration of 0.3M, 10μmoles $MgCl_2$, 20μmoles GSH, 50μmoles Tris-HCL(ph 7.5) (at 25°), 2μmoles dipotassium ATP adjusted to pH 7.5 with KOH, 10μmoles creatine phosphate, 120μg creatine kinase, 0.5μmoles GTP, a mixture of 20 amino acids, each 5μmoles, and 0.5μC of a mixture of 13*L*-[^{14}C] amino acids. The mixtures were incubated at 37° for 1 h.

Nature of Product of Protein Synthesis

The characterization of the soluble product of the cell-free system was accomplished in several ways.[7,8] When carrier contractile protein (platelet actomyosin) was added to the dialyzed supernatant fraction of the incubation mixtures, some 40% of the total radioactivity was recovered in the reisolated contractile protein. This soluble synthetic product had solubility properties similar to those of the carrier contractile protein and "fingerprint" identical to that of contractile protein.

The synthetic product was further studied by analysis on a discontinuous sucrose gradient. Platelet contractile protein is composed of a myosin-like and actin-like moiety that can be separated on a discontinuous sucrose gradient (Fig. 1). A similar pattern is obtained when purified contractile protein is treated with ATP and $MgCl_2$ before layering on the gradient. Control experiments were performed with purified ^{14}C-labeled contractile protein (isolated from intact platelets) that had been treated with ATP and $MgCl_2$ immediately prior to centrifugation on the gradient. Radioactivity was associated with both the myosin (Fig. 1, *peak 1*) and actin (Fig. 1, *peak 2*) moieties. When such an experiment is carried out on either the reisolated carrier contractile protein or directly on the dialyzed supernatant fraction of the cell-free system, radioactivity was similarly associated with both the platelet myosin and platelet actin (Fig. 1). The high percentage of recovery of radioactivity (40%) from the soluble protein fraction as contractile protein and the similarities in sucrose density gradient and fingerprint patterns of the synthetic product and contractile protein indicate that contractile protein is a major soluble product of the cell-free system obtained from human platelets.

A similar conclusion was reached by analysis of the product of protein synthesis by intact platelets. In this case 65 to 70% of the total incorporated radioactivity was recovered as contractile protein. The sucrose density gradient and "fingerprint" patterns of this synthetic product were identical with those of the product of the cell-free system and purified contractile protein.

An analysis of the soluble protein fraction indicates that some radioactivity may have been incorporated into albumin, fibrinogen, and a protein which binds thrombin.* In this case it is not

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*Unpublished observations.

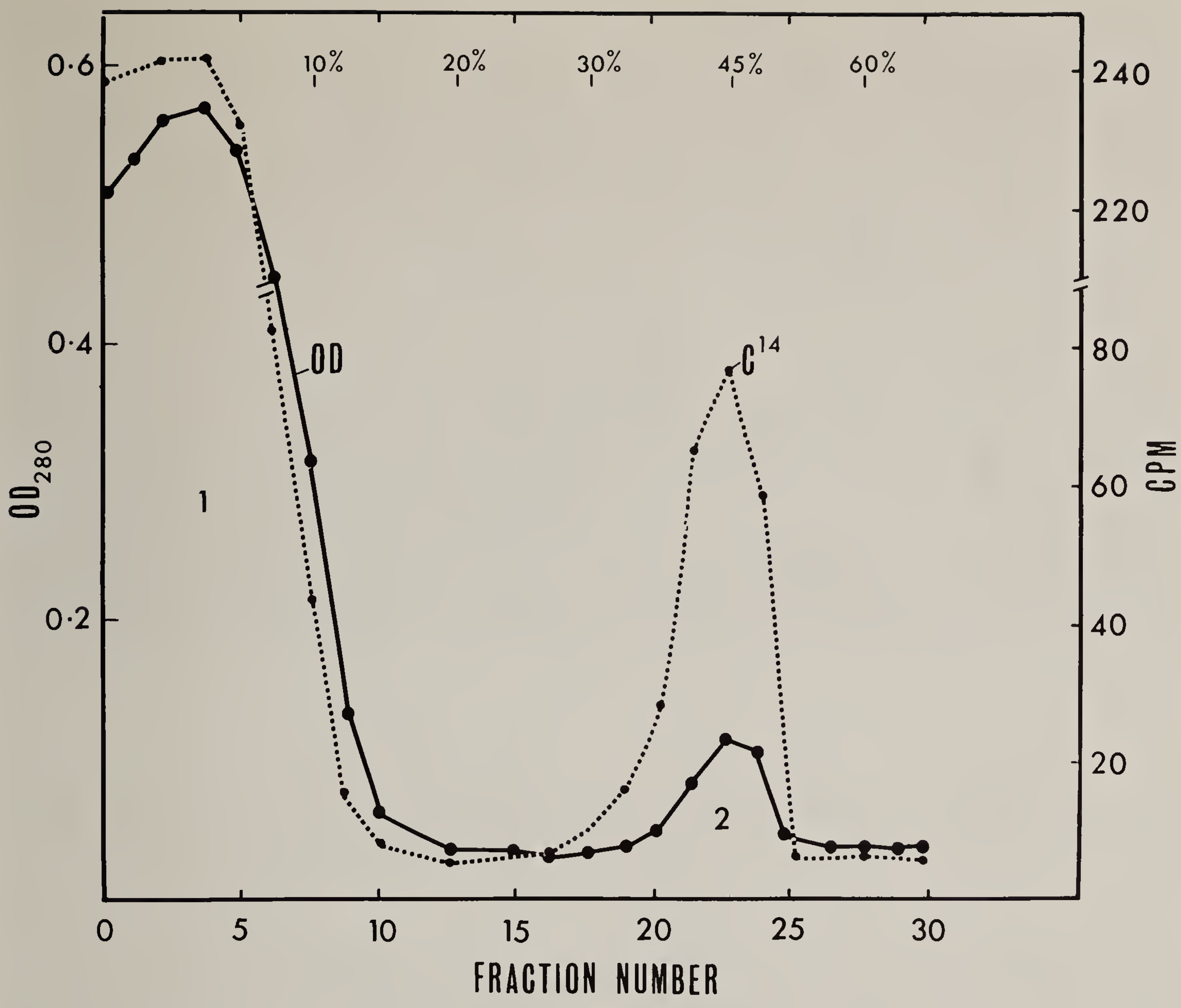

Fig. 1.—Discontinuous sucrose gradient pattern of ^{14}C-labeled cell-free system product treated with 5mmolar ATP and $MgCl_2$ immediately before centrifugation at 25,000 RPM for 8 h. (*1*) platelet myosin and (*2*) platelet actin.

known whether this represents merely nonspecific N-terminal addition of amino acids or indicates total synthesis. In the case of contractile protein the incorporation of amino acids throughout the entire molecule has been demonstrated.[2,7]

Cellular Location of Synthesized Protein

The synthesis of protein by platelets suggests that this activity might have an active role in platelet function. A prerequisite for understanding any possible role of the protein synthetic product is a knowledge of its specific location within the cell. This was attempted in intact platelets in a series of experiments employing pulse-labeling and autoradiographic techniques with labeled amino acids.[8,9] It may be seen from the electron microscope autoradiographs shown in Fig. 2 that a surface or near surface location is indicated for the protein synthesized at all pulse-labeling times. The question arises, however, whether this protein is actually localized at specific sites, as indicated in Fig. 2, or whether the protein is distributed over the platelet surface. The specific sites indicated may merely represent the initial stages of the distribution process of the labeled protein over the platelet surface. In an attempt to answer this question, pulse-labeled platelets were "chased" with un-

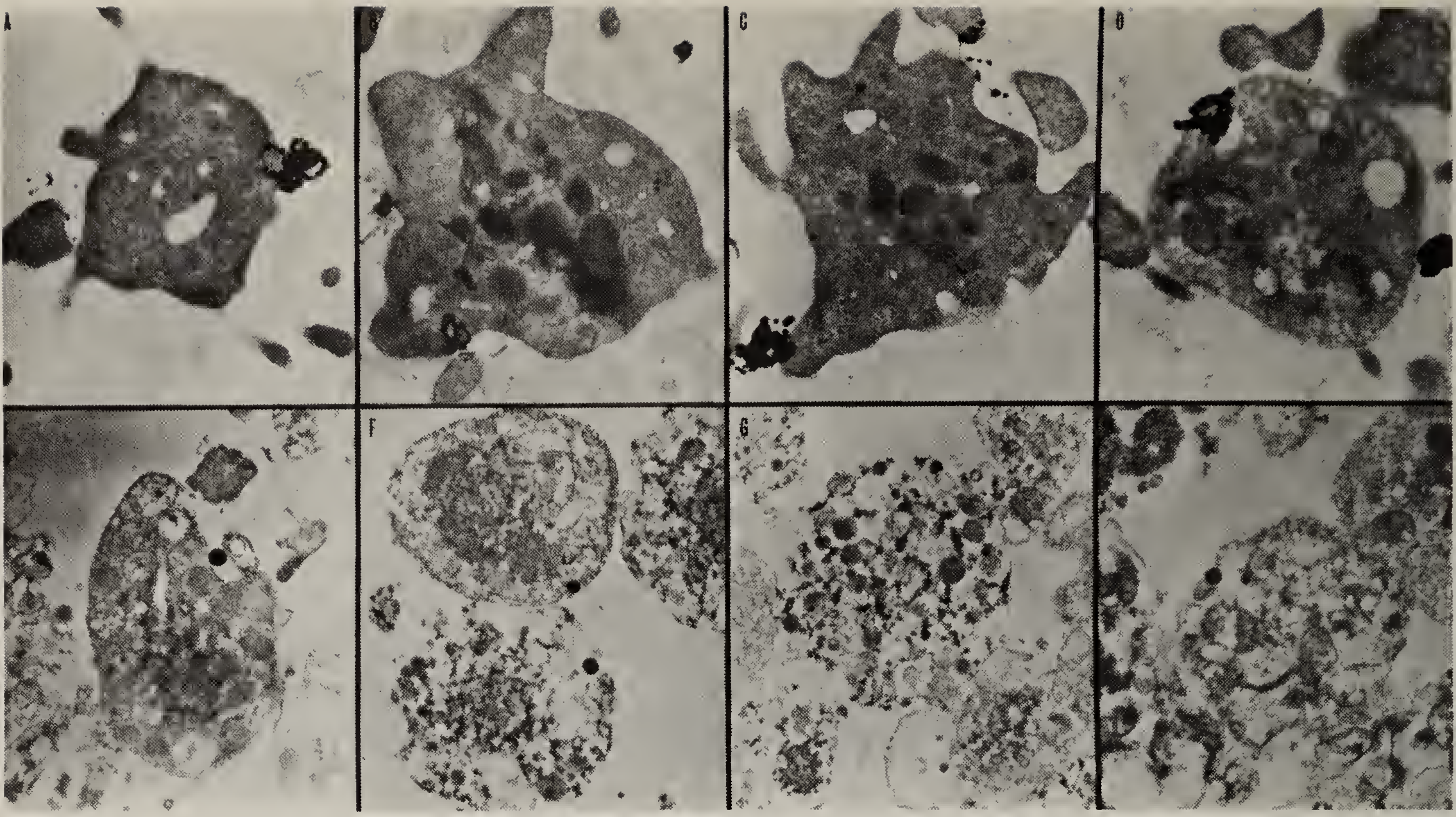

Fig. 2.—Electron microscope autoradiographs of ^{3}H-leucine (top row) and ^{14}C-amino acids (bottom row) labeled platelets; *A* and *E* pulse labeled for 5 min; *B* and *F*, 15 min; *C* and *G*, 60 min; and *D* and *H*, 120 min.

labeled amino acids in order to provide a time interval for the possible relocation of the protein on the platelet-surface. In no instance, however, could a spreading or redistribution of radioactivity be observed on the autoradiographs. All autoradiographs obtained after chase experiments still showed specific site locations. One or two exposure sites were usually seen, and in rare instances three sites. These results, in conjunction with other data to be discussed, indicate that the labeled protein (primarily contractile protein) is indeed localized in specific sites on the cell surface. A subsequent mathematical analysis of the average number of labeled sites observed in 200 platelet autoradiographs in relation to the surface area and number of sections obtained from an average-size platelet indicate that the labeled protein must be localized at specific sites near or on the surface and could not be distributed over the entire surface of the platelet.

It was further shown[8,9] that contractile protein could be extracted from platelets while leaving them intact. Platelets so extracted and still intact cannot be induced to aggregate by any conventional agents. It was also shown by sucrose density gradient analysis that the membranes from such extracted platelets were significantly *lighter* than those from non-extracted platelets, indicating that some heavier component(s) had been removed. Furthermore, contractile protein was recovered from the extract in amounts comparable to that now missing from the membranes. Knight *et al*[10] have reported a similar extraction of an actomyosin-like protein from the membrane of embryo-chick fibroblast cells; these treated cells also could not be induced to aggregate under their experimental conditions. Additional evidence for the surface location of at least part of the platelet contractile protein has been provided by other investigators.[11,12] Liver cells,[13] yeast cells,[14] and slime mold plasmodia[15] have also been reported to have an actomyosin-like contractile protein at their limiting membranes.

A Contractile Protein Model for Platelet Aggregation

The molecular model for platelet aggregation we have proposed[16] requires the direct participation of a surface localized actomyosin-like contractile protein. The evidence for the surface location of the platelet contractile protein and its participation in the aggregation process was presented in the previous section. By *aggregation* we mean the phenomenon whereby platelets adhere to each other. Adhesiveness refers to the property of platelets to adhere to a foreign surface, and agglutination refers to clumping due to an antibody-antigen reaction.

The following mechanism, based upon the known properties of contractile proteins, would seem to accommodate and provide a basis for explanation of the reactions involved in platelet aggregation:

(a) A surface or near surface localized (exposed to environment) platelet actomyosin-like contractile protein catalyzes the splitting of ATP.

$$\text{ATP} \rightarrow \text{ADP} + \text{Pi}.$$

The concentration of available substrate (ATD) is, however, below the critical concentration required to dissociate the platelet actomyosin complex into its component parts, platelet F-actin and platelet myosin.[17]

(b) Under the influence of a physiologic stimulus such as thrombin, which stimulates glycolysis[18] the concentration and availability of ATP is greatly increased. The ATP concentration is increased to that required for dissociation, resulting in the dissociation of the platelet actomyosin complex into platelet F-actin and linear aggregates of platelet myosin.[19]

(c) In the dissociated form the ATPase activity is low, *i.e.*, that of platelet myosin alone.[17] As the ATP concentration is decreased due to the ATPase activity of the platelet myosin, reassociation of the dissociated moieties begins and the ATPase activity rises as the MgATPase (or CaATPase) of the platelet actomyosin complex evidences itself.

(d) This reassociation, however, does not merely involve *intermolecular* interaction between protein moieties on a single platelet, but extensive *intermolecular* interaction between platelet F-actin and platelet myosin on adjacent platelets.

(e) These *intermolecular* platelet actomyosin bridges are then able to contract in the presence of ATP (at concentrations below that required for dissociation of complex), drawing the platelets into a tight mass or thrombus.

A schematic illustration of the model is given in Fig. 3.

According to the proposed model, the driving force in platelet aggregation is the reversible dissociation of a platelet actomyosin-like complex exposed to the environment. Conditions that effect the interaction and dissociation of contractile proteins have been well documented.[20] A definite set of interrelated, well-defined conditions are required to maintain the integrity of the actomyosin complex. It is therefore conceivable that any reactions and/or factors interfering with the steady state conditions required to maintain the integrity of this complex will result in alterations in the interaction process and subsequently be reflected by alterations in platelet adhesiveness.

For example:

(1) The concentration of free calcium has a key role in the actin-myosin interaction process. Several reports have indicated that MgATP is the moiety that controls the dissociation of actin and myosin by altering the level of calcium needed for association.[21,22] Removal of either calcium and/or magnesium with chelating agents such as EDTA, citrate and oxalate would result in dissociation of the actomyosin complex, thereby preventing the formation of *intermolecular* platelet actomyosin bridges and aggregation.

(2) Sulfhydryl blocking reagents have the ability, not only to block ATPase activity, but also -SH groups required for actin-myosin interactions.[20,23] These blocking agents would be expected to prevent or inhibit aggregation due to the inability to form *intermolecular* protein bridges.

(3) Addition of ADP would result in

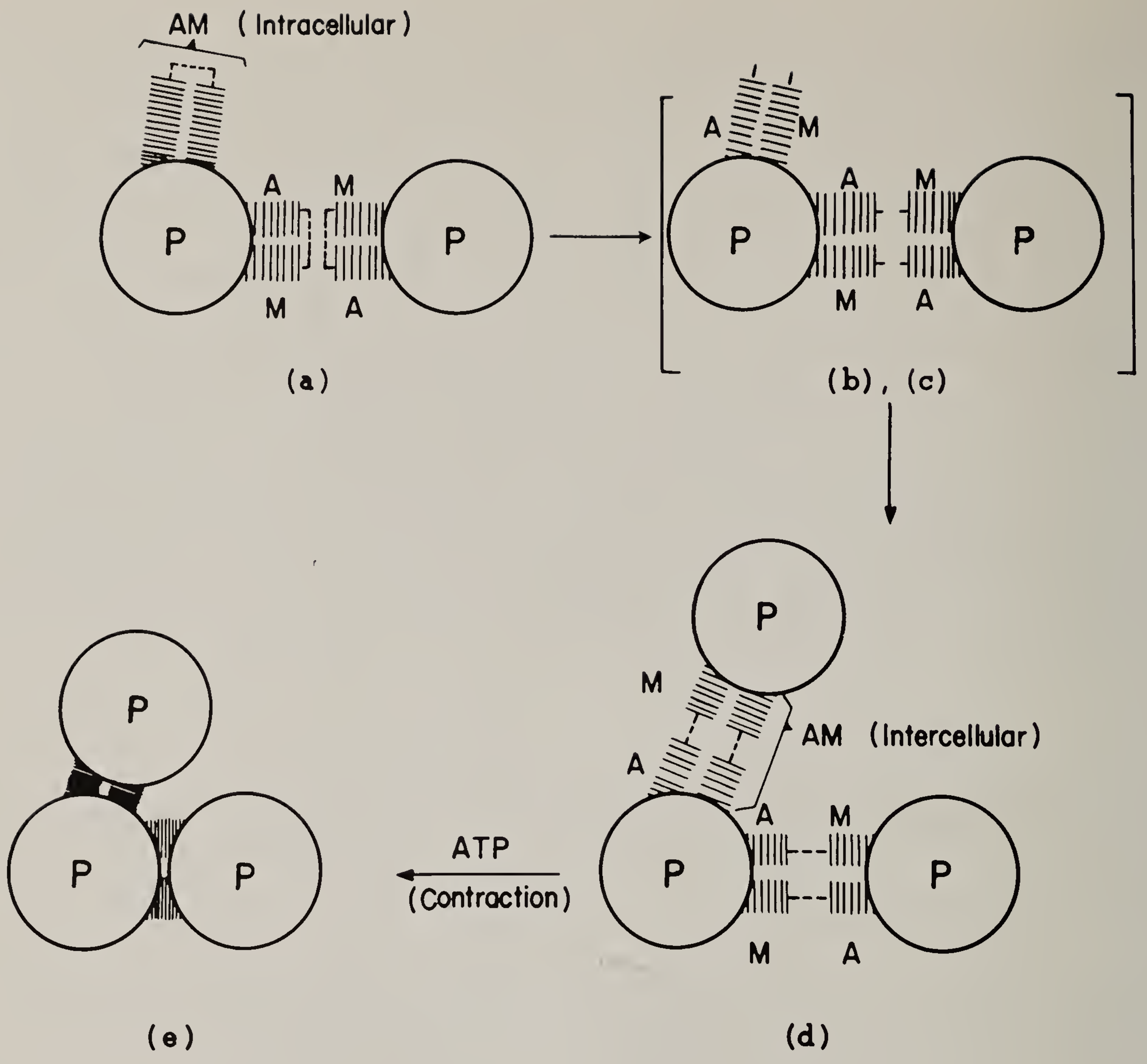

Fig. 3.—Schematic illustration of model system: (a)–(e) represent various stages in the aggregation process as described in text; *AM*, platelet actomyosin; *A*, platelet F-actin; *M*, platelet myosin; *P*, platelet.

competitive inhibition of the enzyme reaction (ATPase activity)[12,24] causing an accumulation of ATP substrate and subsequent initiation of both the dissociation and aggregation processes.

(4) Finally the addition of ATP to actomyosin solutions causes a marked decrease in both viscosity and intensity of light scattered by the actomyosin solutions. These decreases have been associated with the dissociation of the actomyosin complex into its component parts. ATP acts here as a competitor with actin for combination with myosin. Born *et al.*[25] have shown that a large number of adenosine analogues are active as inhibitors of aggregation. Analogues belonging to this group, as well as analogues described by Ikehara *et al.*,[26] however, have been shown to decrease drastically the viscosity and intensity of light scattered by the treated actomyosin solutions. This, presumably is due to dissociation of the actomyosin complex by the specific analogues. Related compounds such as AMP and adenosine could have a similar dissociating ability, thereby inhibiting platelet aggregation.

The proposed model provides a molecular basis for the attachment of one platelet to another, is consistent with the general reactions and factors involved in platelet aggregation, and appears to provide a reasonable explanation for the data available at this time.

It is of considerable interest that an antigenic relationship between blood platelets and vascular endothelium has recently been demonstrated by Morrison and Baldini.[27] The contractile protein may be one of the common antigens, as there is some evidence for its presence in the endothelial cell.* If this indeed is so, then one could postulate a similar molecular mechanism for the attachment of the platelet to a site of injury on the vascular endothelium.

Platelet Populations

Autoradiographic and electron microscopic studies with normal random platelet populations indicated a heterogeneity in the size and morphology of platelets, in the content and distribution of subcellular elements, and in their ability to incorporate labeled amino acids. These observations indicated that platelets did not exist as a homogenous population and that it might be possible to separate platelets into more homogenous populations. Such a separation into four populations was subsequently achieved by centrifugation on a discontinuous sucrose gradient.[8,28,29] Similar separations into various populations have also been obtained with dog and marmoset platelets. A typical normal pattern for the human is shown in Fig. 4*a*, in which band D contains the platelets of highest density.

The typical effects of inhibition and induction of thrombopoiesis on the platelet distribution pattern are illustrated in Fig. 4*b* and 4*c* respectively. Suppression

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*Unpublished observations.

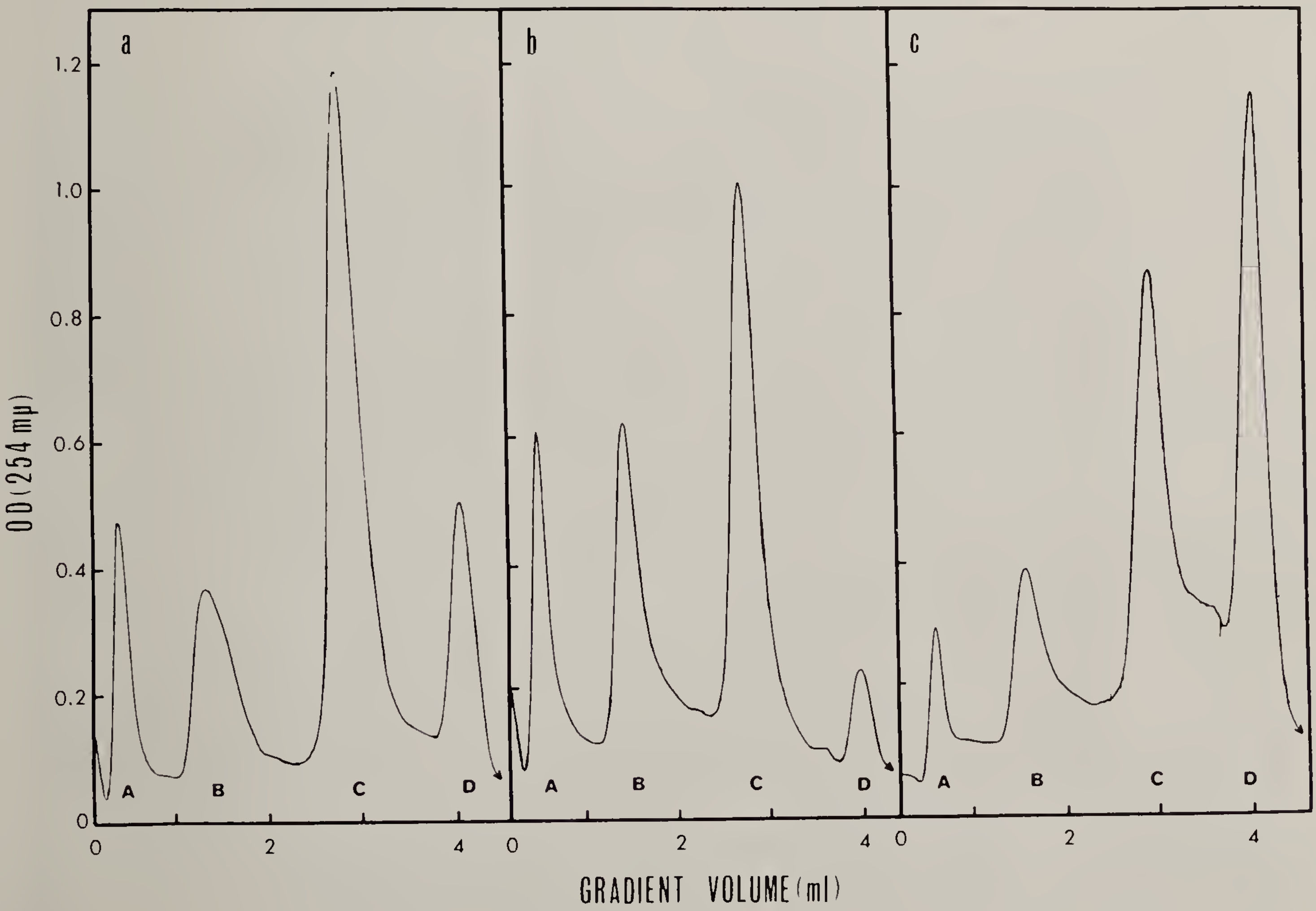

Fig. 4.—Separation of human platelets into four populations (*A, B, C, D*) on a discontinuous sucrose gradient. Arrows indicate bottoms of gradients. *a*. Normal individual. (Platelet count: $3 \cdot 5 \cdot 10^5$.) *b*. Individual undergoing immunosuppressive therapy (post renal transplant) in which platelet formation was depressed with azathioprine (Imuran). (Platelet count: $9 \cdot 10^4$.) c. Individual with chronic thrombocytopenic purpura in which platelet formation was induced with normal plasma. (Platelet count: $5 \cdot 5 \cdot 10^5$.)

of platelet production was obtained in renal transplant patients treated with high doses of the immunosuppression drug, Azathioprine. The decrease in band *C* and band *D* platelets (Fig. 4*b*) was associated with a decrease in the average platelet count from $3.5 \times 10^5 mm^3$ to $9 \times 10^4 mm^3$. Formation of new platelets was induced in a patient with chronic thrombocytopenic purpura by transfusion with small amounts of normal plasma.[30] When compared to the distribution pattern obtained prior to transfusion, the pattern obtained following induction (Fig. 4*c*) showed a marked increase in platelet count from $2 \times 10^4/mm^3$ to $5.5 \times 10^5/mm^3$. Platelet distribution patterns of post-surgical patients with transient thrombocythemia were very similar to the distribution pattern obtained after induction of thrombopoiesis (Fig. 4*c*). We believe, on the basis of these results, that young or newly formed platelets are represented by bands *D* and *C*.

Characterization of Platelet Populations

The separated platelet populations were characterized under the electron microscope. A composite electron micrograph of platelets from each of the four bands is shown in Fig. 5. The most pronounced morphologic differences are found in comparing platelets from band *A* (top of the gradient) with those in band *D* (bottom of the gradient). Band *A* is composed almost entirely of platelet membranes, fragments of platelets, and extensively damaged platelets containing very few or no granular structures. In contrast, band *D* consists of intact platelets of uniform size and shape containing numerous granular structures. Bands *B* and *C* appear to represent intermediate stages between the morphologically distinct populations in bands *A* and *D*. Band *C* appears the most heterogeneous of the four populations and contains large and bizarre-shaped cells in addition

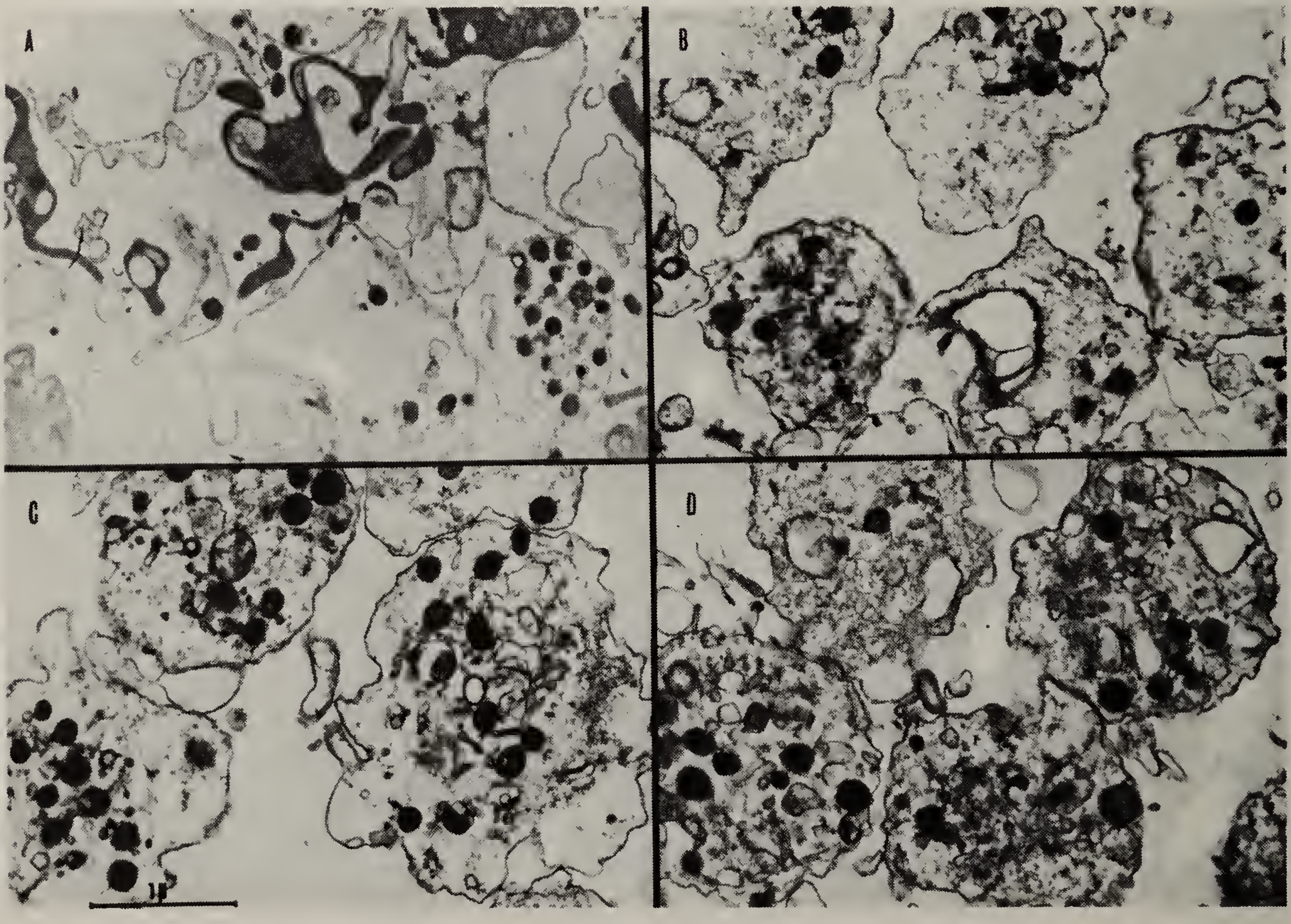

Fig. 5.—Electron Micrographs of Platelet Populations (*A, B, C,* and *D*). Separated on a Discontinuous Sucrose Gradient.

to some resembling those found in band *D*. It should be noted that all platelets, regardless of whether they represent bands *A*, *B*, *C* or *D*, will be totaled in the usual platelet count.

Platelets obtained from the individual fractions were incubated with labeled amino acids to examine their individual abilities to carry out protein synthesis. The apparent results shown in Table 3 show that only platelet band *D*, and hence only 20% of the total platelet population, was active in protein synthesis. Although the specific activity (CPM/mg protein) of band *D* is appreciably higher than that of band *C*, autoradiography showed that the highest grain counts per exposure area were located in *some* of the platelets of band *C*. This indicates that some of the band *C* platelets are highly active, and perhaps the most active in protein synthesis. This also affords additional evidence for the heterogenity of this platelet population. Band *D* platelets, followed by band *C* platelets, have the highest ATP content (Table 3), are most richly endowed with enzymes* and are the most active in aggregation.[8,28,29] The content of ATP has been reported to decrease with aging,[31] and Wright[32] reported many years ago that newly formed platelets were "stickier" than those normally present. We have found that band *D* platelets, when maintained *in vitro*, undergo marked density changes resulting in rapid shifts to bands *B* and *C* and eventually band *A*. We conclude from all of these data that band *D* platelets (and perhaps some platelets in band *C*) represent young or newly formed platelets and are the most biochemically competent and morphologically intact platelets. Bands *A* and *B* and the majority of cells in band *C* represent damaged platelets and perhaps the older populations. These observations may be interpreted in terms of the suggested messenger RNA being degraded with time, which in turn may result in the gradual shift to "older" platelets of lower density with decrease or absence of protein synthesis ability. This system may be analogous to the mammalian reticulocyte-erthrocyte system, in which only the reticulocyte synthesizes protein, this ability being lost in mature erthrocytes. Steiner and Baldini[6] have recently provided additional experimental support for our conclusions. In their experiments platelet cohorts were produced in rats following immunologically-induced thrombocytopenia. Young platelets had a significantly greater rate and magnitude of amino acid incorporation into protein than aged ones. They interpret this to indicate a maturation process in platelets similar to that in reticulocytes.

TABLE 3. WEIGHT DISTRIBUTION, ATP-CONTENT, AND AMINO ACID INCORPORATION OF PLATELET POPULATIONS

Population	% Weight Distribution*	ATP-Content μM/mg Protein	Amino Acid Incorporation Counts/min per mg Protein
A	16	0.7	5
B	19	1.0	13
C	45	2.8	52
D	20	3.8	263

*Percentage of total weight applied to gradient.

It may be that the life span of the platelet is determined in part by its ability to carry out protein synthesis, although we have no data to support directly this contention. We have no data to indicate a physiologic turnover of contractile protein and no information on control mechanisms that might exist. We must consider the possibility that some platelet diseases may be due to either a failure to synthesize adequate amounts of contractile protein (or some other protein) or to making a "wrong" protein. With our current methodologies we can begin to probe such questions.

Some Practical Applications of Separation of Platelet Populations

The remarkable constancy of platelet distribution patterns in normal individuals (Fig. 4*a*) led us to investigate the application of the density gradient technique in studying clinical situations in which platelet abnormalities have been described or suspected. Preliminary results have indicated that a number of clinical disorders with bleeding tendencies were associated with alterations in the distribution of platelet populations. To illustrate the application of this technique we will consider only patients with uremia and polycythemia vera in which bleeding tendencies have been well documented.[32-34]

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*Unpublished observations.

Uremic patients (before hemodialysis) with a normal platelet count have a markedly altered platelet distribution pattern as is shown in Fig. 6*b*. Compared to the normal pattern (Fig. 6*a*), it is evident that bands *C* and *D* are virtually non-existent and that there is a significant elevation in bands *A* and *B* representing membranes and damaged platelets. It is interesting to note that hemodialysis of these patients restored the distribution patterns to normal, with a simultaneous decrease or disappearance of bleeding tendencies.

An alteration in platelet distribution similar to that found in uremia was observed in patients with polycythemia vera (Fig. 6*c*). Again the most significant feature was the marked increase in bands *A* and *B* and a decrease in bands *C* and *D*.

In the cases studied, bleeding tendencies were associated with altered distribution patterns in which platelet bands *C* and *D* were diminished or virtually absent, and platelet bands *A* and *B* were markedly elevated and represented almost the entire platelet population. Although platelet counts were within the 1.9x10⁵

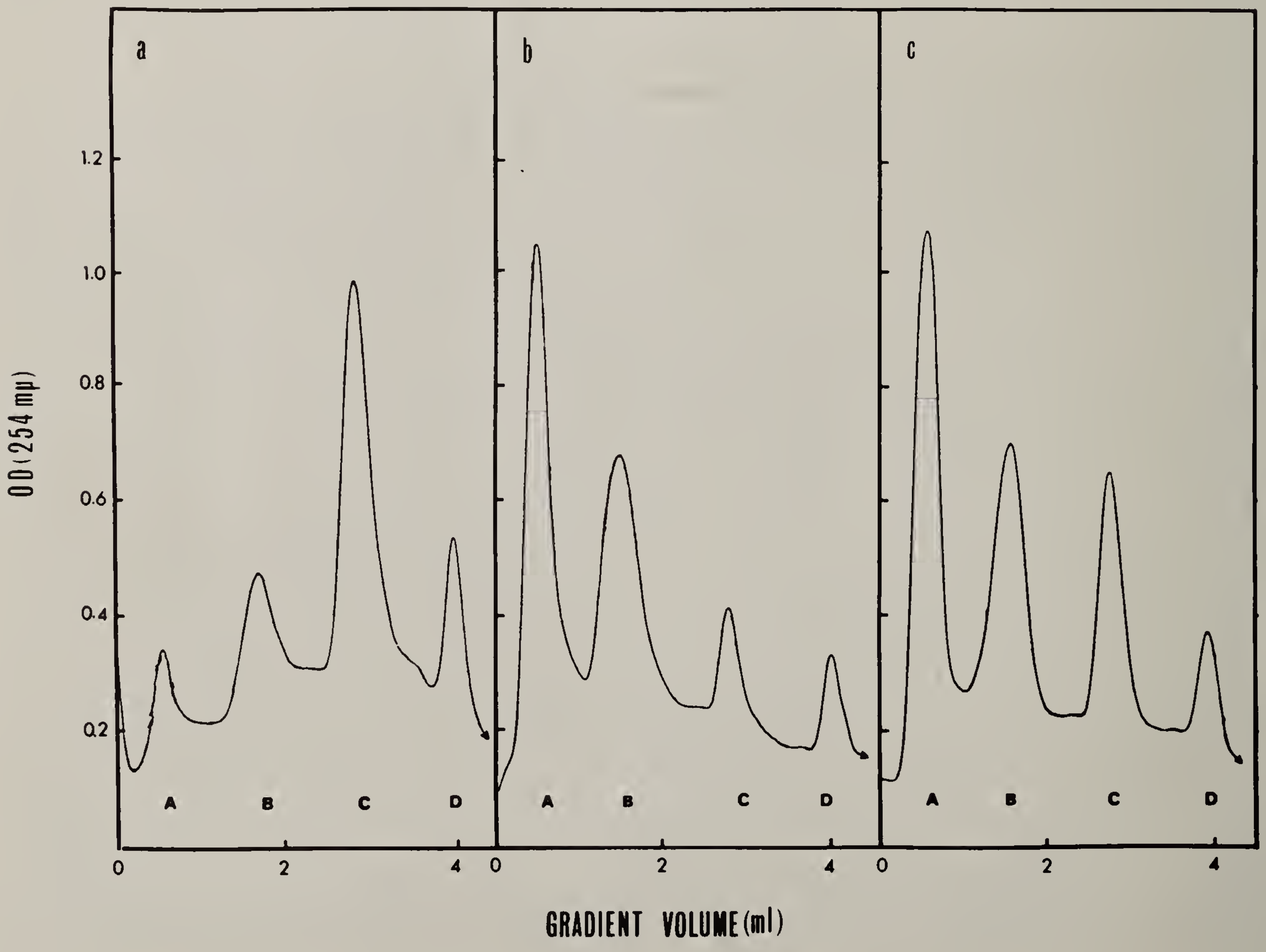

Fig. 6.—Platelet Distribution Patterns Found in Some Disorders with Associated Bleeding Tendencies. *a*. Normal. *b*. Uremia. *c*. Polycythemia vera.

to $4.5x10^5/mm^3$ range in the cases studied, density gradient analysis showed very definite qualitative alterations. These qualitative alterations reflect changes in platelet density due to changes in size, shape, protein content, granulation, and metabolic activity. Platelet bands *C* and *D* appear to be important in the hemostatic process and may be required in sufficient amounts or ratios to prevent bleeding. Bands *A* and *B* may represent platelets with little or no ability to maintain normal hemostasis and therefore may be the major cause of the observed bleeding tendencies.

Although the platelet distribution patterns in uremia, polycythemia vera, and other disease states are reproducible, it should be emphasized that the pathophysiologic and diagnostic significance of such alterations remain to be ascertained. Analysis of platelet distribution patterns in conjunction with platelet function studies and changes in platelet counts may provide additional information on the clinical significance of qualitative and quantitative platelet defects.

The separation of platelet populations by density gradient centrifugation has some other potentially useful applications. Among others, these are the study of *in vitro* storage conditions for platelets, the possible effects of various drugs (Fig. 7), and the effects of various antibodies (Fig. 8) and aggregating agents (Fig. 9) on the distribution patterns. We present these patterns at this time without detailed comment simply to show some potential applications of the technique and to encourage its use by others.

Some Concluding Remarks

The hitherto neglected blood platelet has apparently provided us with a number of new and somewhat unexpected areas for continued biochemical investigation. At this time our conclusion as to the significance in platelet function of protein synthesis, the contractile protein, and platelet populations must be con-

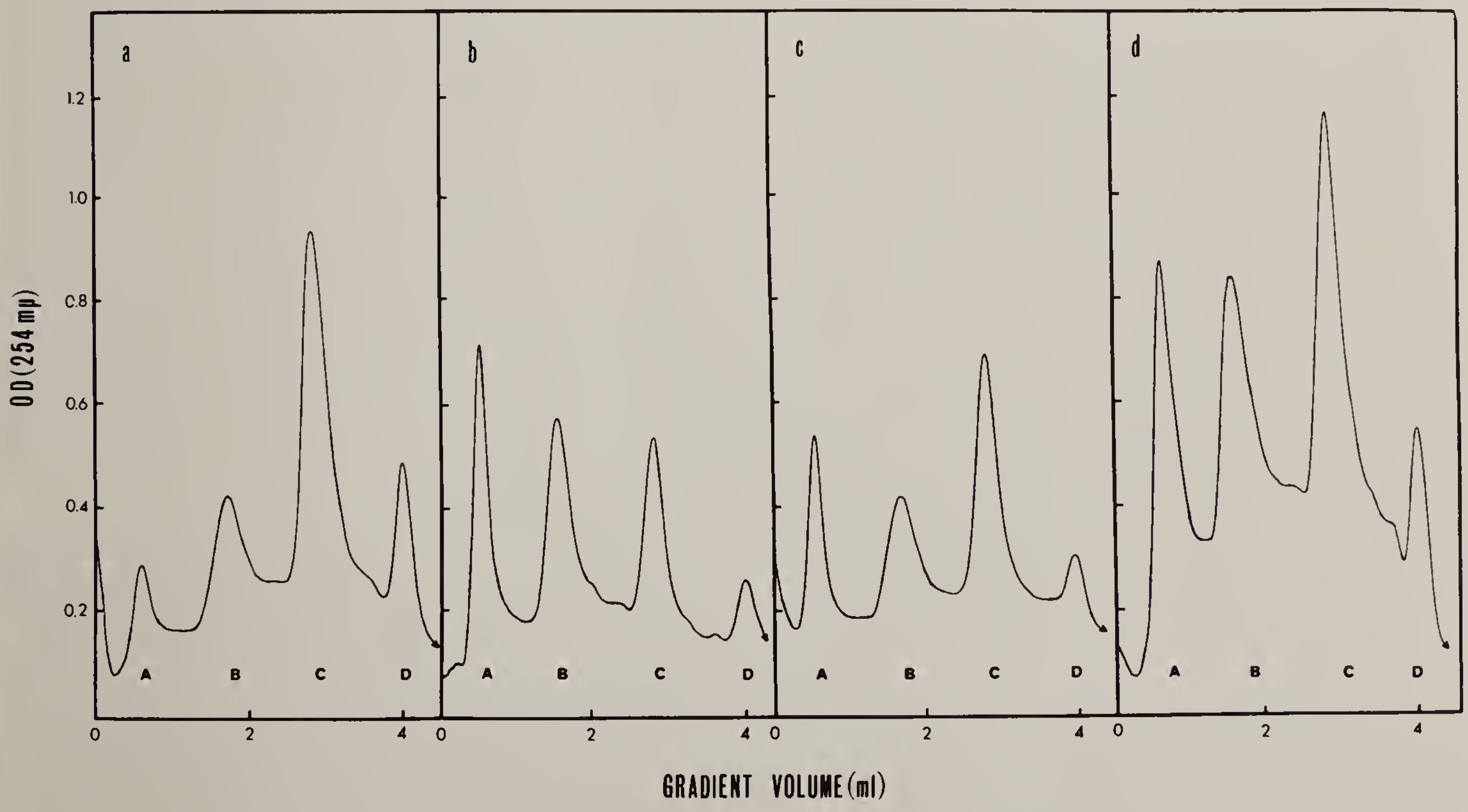

Fig. 7.—Effects of Various Drugs on Distribution Patterns of Platelet Populations. *a.* Normal individual. *b.* Post renal transplant patient undergoing immunosuppressive therapy with azathioprine. c. Cancer patient treated with mithramycin. *d.* Aspirin.

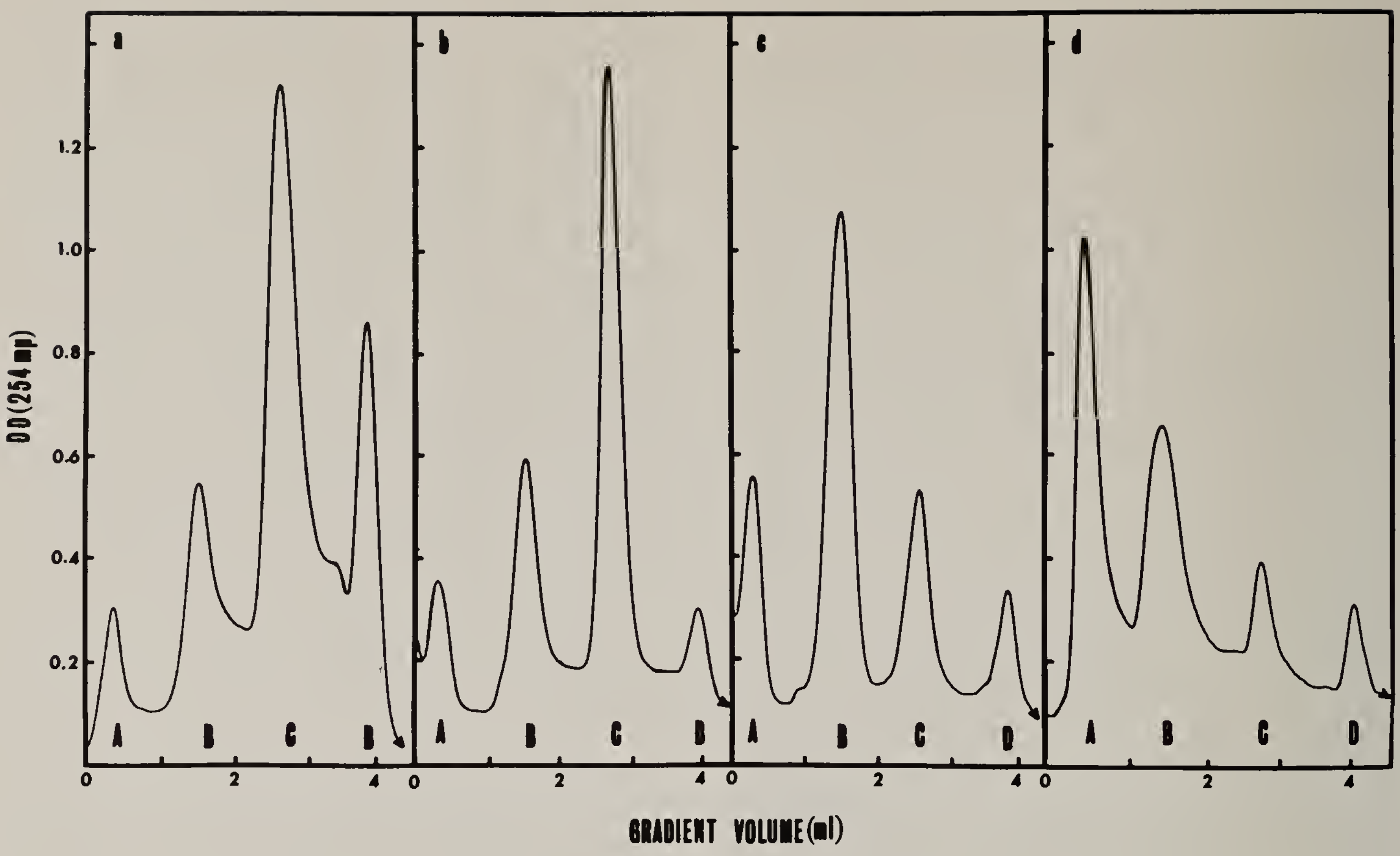

Fig. 8.—Effects of Various Antisera on Distribution Patterns of Platelet Populations. *a.* Incubated control. *b.* Antiplatelet serum. *c.* Anti-lymphocytic serum (unabsorbed for platelet components). *d.* Antiplatelet serum.

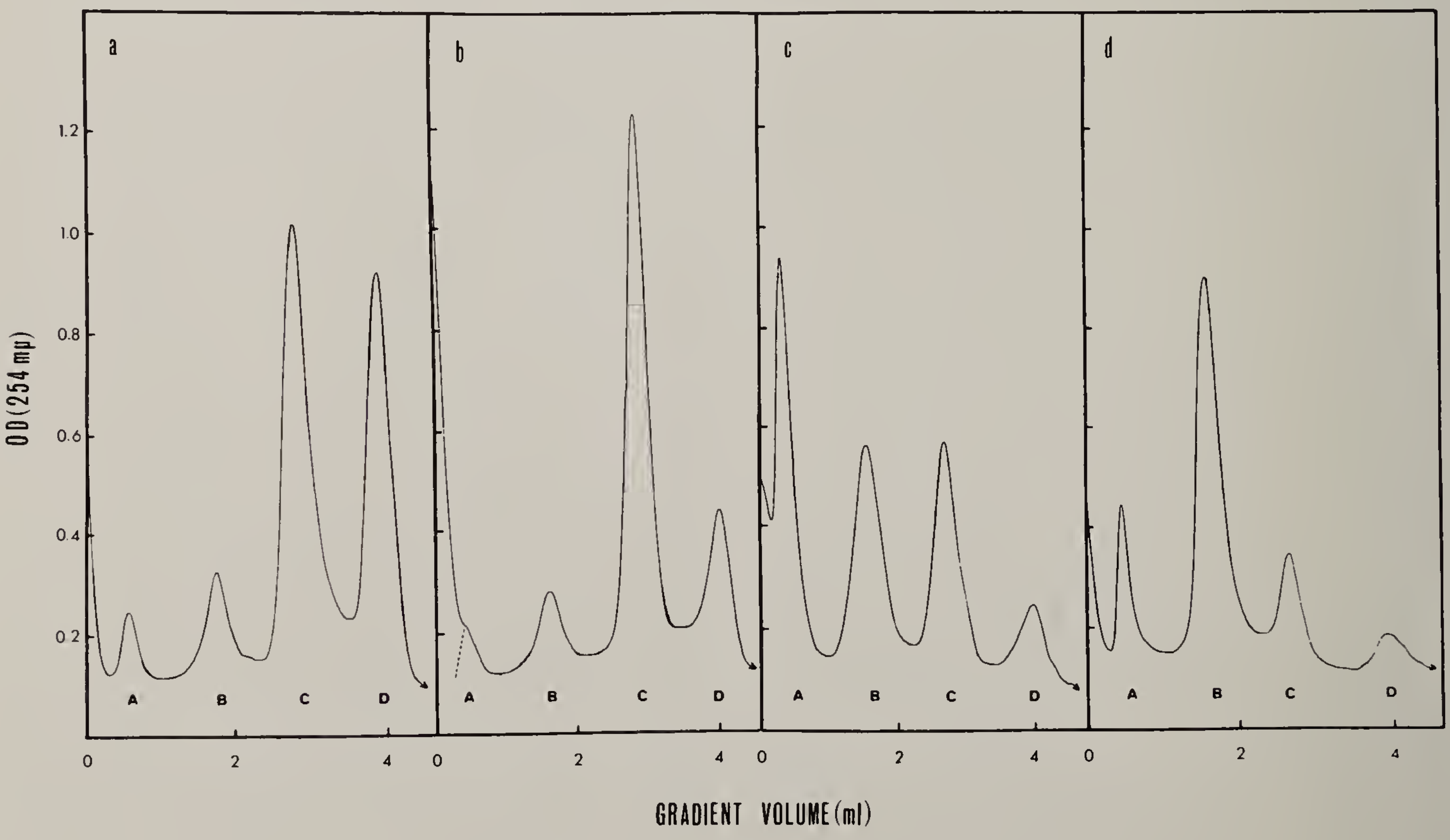

Fig. 9.—Effects of Various Platelet Aggregating Agents on Distribution Patterns of Platelet Populations. *a.* Control; in this case a post-surgical patient with transient thrombocythemia. *b.* ADP. *c.* Collagen. *d.* Thrombin. In these experiments the minimal amount of material to produce visible aggregation was used. As soon as visible aggregation was observed the platelets were applied to the gradients and centrifuged.

sidered speculative. Also, these represent only a portion of worthy platelet problems that need detailed investigation. It is also clear that a real understanding of the role of platelets in hemostasis and thrombosis will require a multidisciplined approach. Not only will much more biochemical data be required, but at least as much information will have to come from physical chemical, clinical-physiological, and morphological approaches. Some lines by J. W. von Goethe aptly sum up our real needs in platelet research:

"Precise design, swift exercise
Will ever win the fairest prize;
To make the splendid dream
come true,
One mind for thousand hands
will do."

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REVIEW ARTICLE

CONGENITAL ANOMALIES OF THE CORONARY ARTERIES

John A. Ogden

The congenital anomalies of the coronary arteries form a varied group whose individual members range in significance from the majority having no clinical manifestations to those whose presence is incompatible with survival beyond birth. Attention to these anomalies has paralleled the increasing numbers of hearts visualized at surgery, observed critically at autopsy, and studied by angiography.[1,2] This paper is a report of the analysis of the records of 182 patients with documented congenital anomalies of the coronary arteries (Table 1). Information reviewed in each case included description of the specific coronary anomaly, associated cardiovascular anomalies, age, sex, symptoms, physical findings, and pertinent laboratory data. In those patients subjected to surgery, the indications for operation and details of the surgical procedure, and the postoperative course were investigated. Roentgenograms, angiocardiograms, cardiac catheterization data, and autopsy protocols were reviewed. For the purposes of studying and presenting the material, the anomalies were organized into three categories as described in 1964 by Blake and his associates: The minor primary anomalies, the major primary anomalies, and the secondary anomalies.[1] A summary presentation of the accumulated information and a general discussion of coronary artery anomalies follows.

The Minor Primary Coronary Artery Anomalies:

The lesions in this group involve variations in the numbers and locations of the coronary artery ostia and in the relatively proximal segments of the coronary arteries, with the distal segments following essentially normal courses. These anomalies cause no physiologic impairment, but they may be difficult to locate and cannulate for angiography and cardiac surgical procedures; they may be injured in the placement of cardiac incisions, and they may influence the course of arteriosclerotic heart disease by altering the pattern of coronary artery obstruction and the development of collateral circulation to the myocardium.

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TABLE I
CONGENITAL DEFECTS OF THE CORONARY ARTERIES

Variations found in transposition complexes	65
Anomalous origin of the left coronary artery from the pulmonary artery	35
Coronary arteriovenous and arteriocardiac fistulas	24
Origin of the left circumflex branch from the right coronary artery	14
Origin of the anterior descending branch from the right coronary artery	10
Single coronary artery	10
All coronary arteries from the same aortic sinus	7
Multiple ostia	6
Anomalous origin of the right coronary artery from the pulmonary artery	3
Hypoplastic and/or atretic arterial segments	3
Extensive mural location	2
Congenital coronary arterial sclerosis	2
Anomalous origin of both coronary arteries from the pulmonary artery	1
Origin of a coronary artery from the posterior (non-coronary) aortic sinus	1
Accessory coronary artery from the left ventricle	1
High take-off from the aortic sinus	1

Normally, there is considerable variation in the location of the coronary ostia within the sinuses of Valsalva. These ostia may be located centrally, they may be shifted laterally toward either adjacent sinus, and they may arise at, above, or below the level of the cusp margins. Occasionally a "high take-off" coronary ostium is located a centimeter or more above the cusp margin, completely out of the sinus of Valsalva. One such case was encountered in this study.

Multiple coronary ostia were seen in six patients in this series. The most common of these anomalies is the accessory conus artery (Fig. 1-*A*), arising from the right aortic sinus by a third ostium and coursing over the right ventricular outflow tract.[3] Less commonly, the anterior descending and circumflex coronary arteries will originate from separate ostia in the left coronary sinus (Fig. 1-*B*).

The origin of the circumflex branch of the left coronary artery from the right coronary artery (Fig. 1-*C*) is found more commonly when there is coexistent transposition of the great vessels than in an otherwise normal heart. Such an abnormally situated vessel may arise directly from the right aortic sinus adjacent to the ostium of the right coronary artery, or it may arise from the main trunk of the right coronary artery close to the origin of the latter vessel from the aorta. From this position it courses behind the great vessels to reach the left coronary artery groove. Similarly, the anterior descending branch of the left coronary artery may arise from the right coronary artery (Fig. 1-*D*) and then course over the right ventricular outflow tract to reach the interventricular groove. In each of these situations of anomalous origin of one of the left coronary artery branches, the remaining branch arises directly from the left aortic sinus.

The mural coronary artery,[4] encountered twice in this study, originates normally from an appropriate aortic sinus, but lies within the myocardium for a part or all of its subsequent course. In one of the cases of this series (Fig. 2-*A*), the left anterior descending branch, just after the bifurcation of the left common coronary artery, dipped into the myocardium and coursed its entire length with 2 to 5 millimeters of myocardial tissue over it. No branches from this vessel appeared on the surface. In the other case (Fig. 2-*B*) the circumflex branch, just after the bifurcation, disappeared into the wall of the left atrium above the left atrial wall, finally emerging on the posterior surface of the heart to give off the terminal branches. Several branches to the left ventricle emerged from the left atrioventricular groove area and pursued epicardial courses.

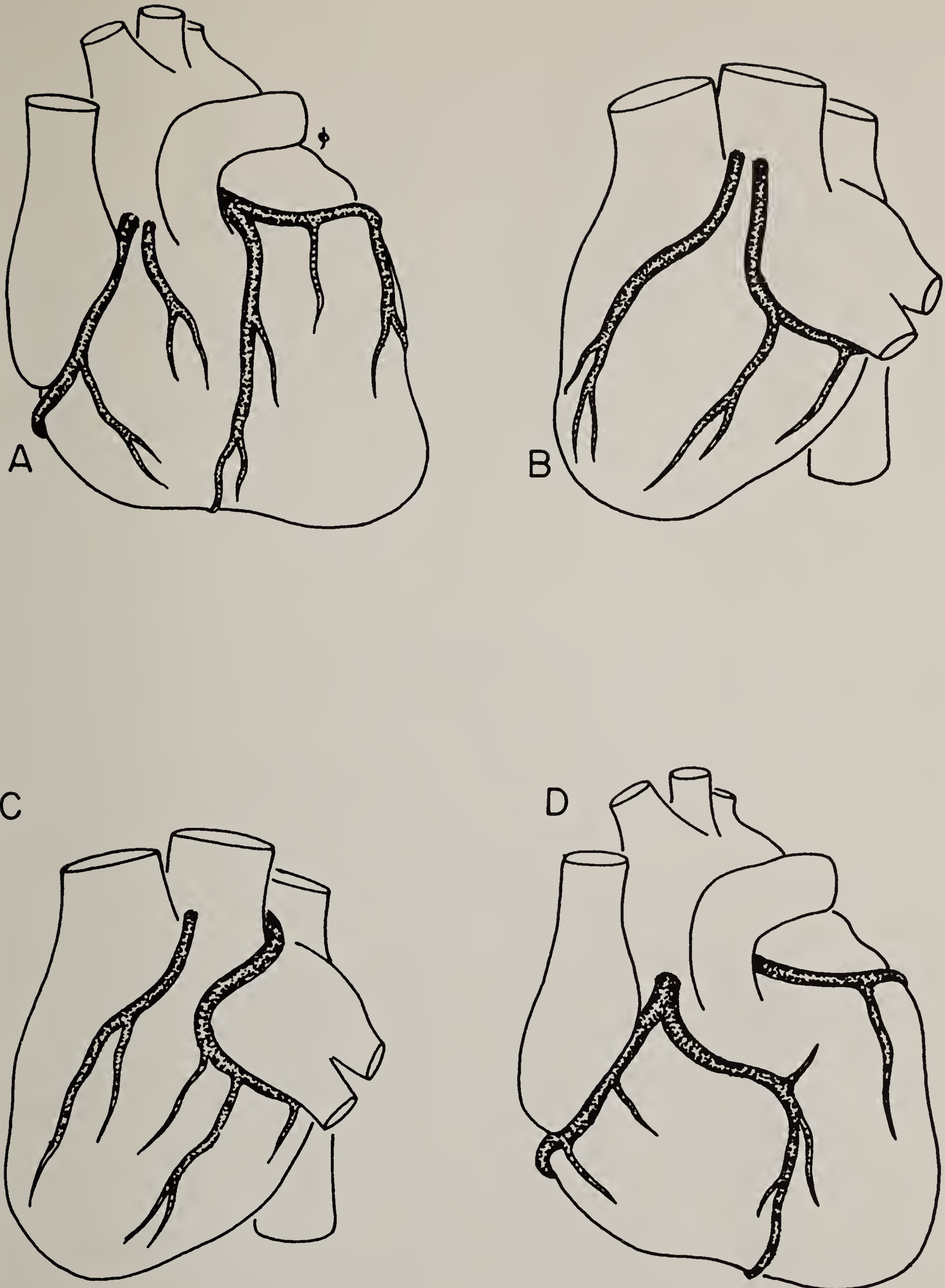

Fig. 1.—Minor primary anomalies. A: separate origin of the conus artery from the right aortic sinus. B: separate origin of the circumflex and anterior descending branches from the left aortic sinus. C: origin of the circumflex branch from the right coronary sinus or artery. D: origin of the anterior descending branch from the right coronary artery.

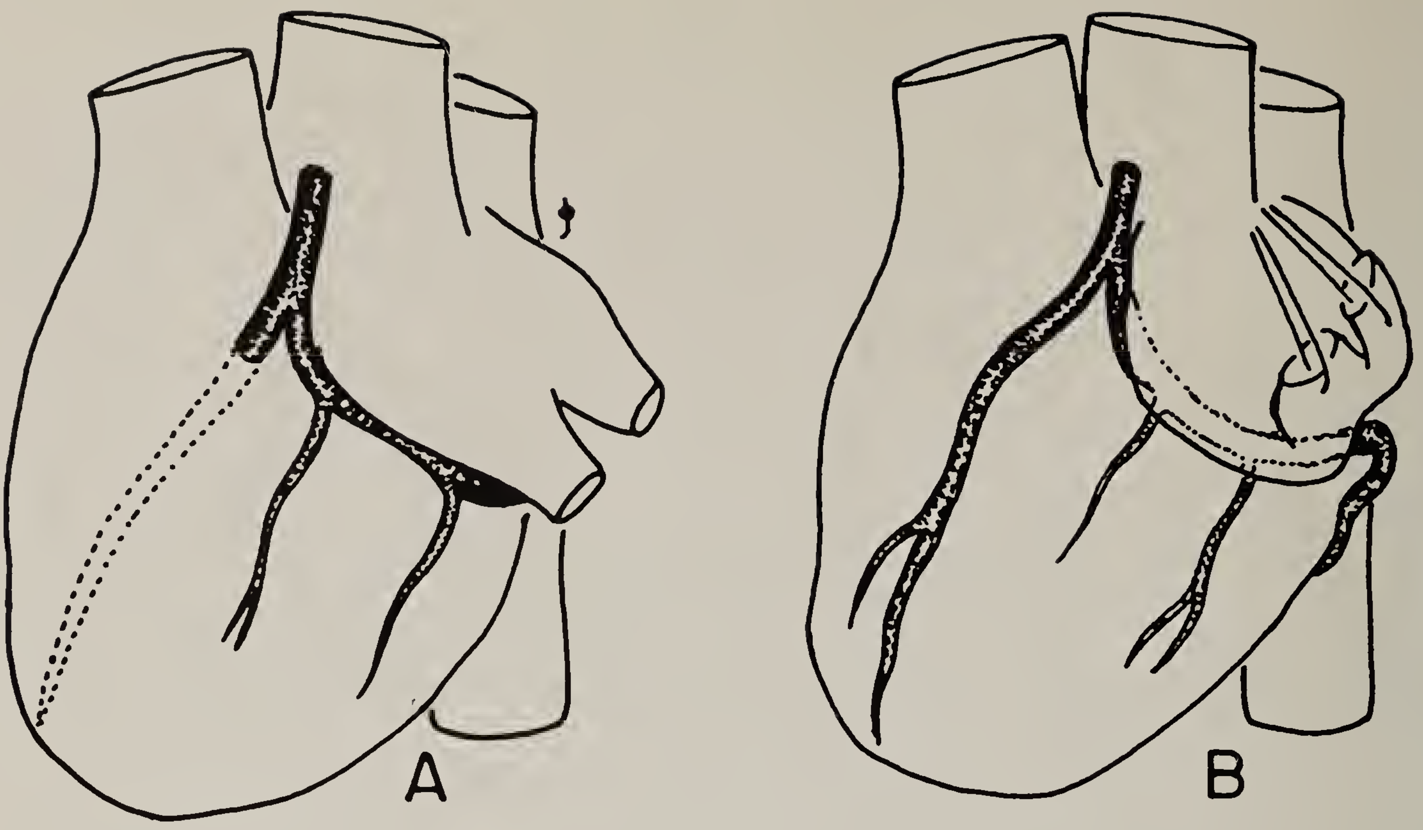

Fig. 2.—Mural coronary arteries. The portion of the artery coursing within the myocardium is indicated by the dotted lines.

The Major Primary Coronary Artery Anomalies:

The anatomic patterns within this group include those of anomalous origin, abnormal communication, and hypoplasia, atresia, or absence of one or both coronary arteries. These anomalies are all physiologically important, some of them being incompatible with life, the remainder being tolerated for varying periods of time from a few hours or days to a normal life span. The lesions of abnormal communication and of abnormal origin of one of the coronary arteries from the pulmonary trunk frequently have the common functional characteristics of an arteriovenous fistula in which the high-pressure coronary arterial system communicates with either a constantly or intermittently low-pressure cardiac chamber or vessel. In addition to their cardiac manifestations, these lesions are capable of manifesting most of the pathophysiology found in the peripheral arteriovenous shunts.

There are two main types of coronary arteriovenous fistulas. The most common type is that in which there is a communication between a coronary artery and the venous side of the heart, including the vena cavae, coronary sinus and tributary veins, right atrium, right ventricle and pulmonary artery. These communications result in a left-to-right shunt demonstrable by cardiac catheterization and angiocardiography. The second type is that in which the communication from the coronary artery is to the arterial side of the circulation, including the pulmonary veins, left atrium and left ventricle. Such a fistula to the pulmonary veins or left atrium functions physiologically in the same manner as a fistula to the vena cavae or right atrium. However, the oxygen saturation differences detected in the left-to-right shunt do not exist in such a left-to-left shunt, and the diagnosis must be confirmed by angiography. A fistula to the left ventricle, shunting only during diastole, is very rare. Representative types of coronary arteriovenous fistulas are illustrated in Fig. 3.

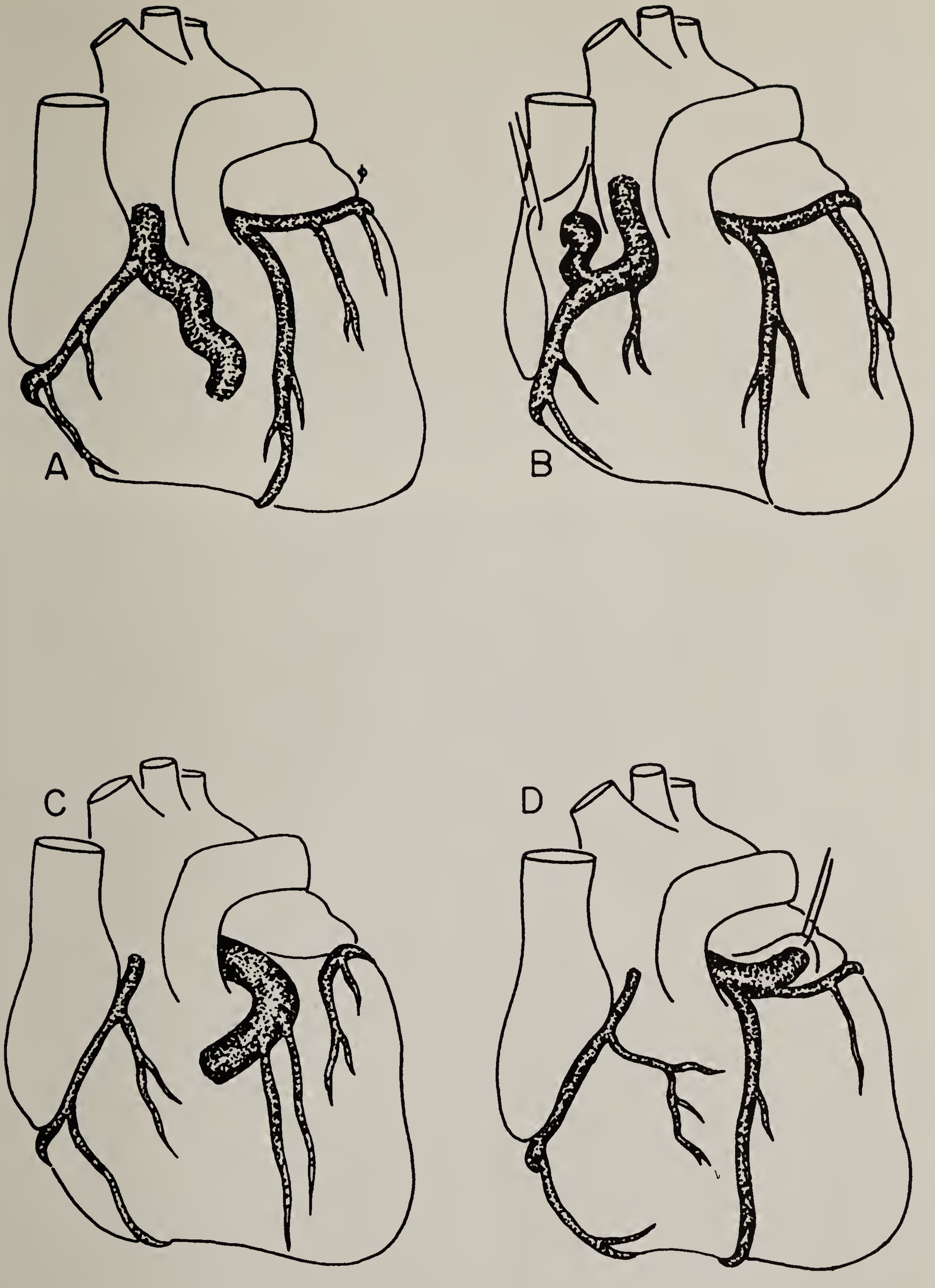

Fig. 3.—Coronary arteriovenous fistulas. A: right coronary artery to right ventricular outflow tract. B: right coronary artery to the right atrium. C: left coronary artery to the right ventricular outflow tract. D: left coronary artery to the left atrium.

The majority of patients with coronary arteriovenous fistulas are asymptomatic or nearly so and are recognized clinically by the presence of a characteristic continuous murmur over the precordium.[5] Occasionally, large fistulas cause congestive heart failure and death. Chest x-rays may show a normal heart or varying degrees of cardiomegaly (Fig. 4). Arteriography shows tortuosity and dilatation of the coronary artery involved in the fistula, and usually demonstrates the shunt into the communicating vessel or cardiac chamber (Fig. 5). Aneurysms of the involved coronary artery are common.

Arteriovenous fistulas emptying into the pulmonary trunk have three anatomic variants. The two coronary arteries may arise normally from the aorta, with an additional vessel arising from the pulmonary artery. There are numerous anastomoses between this anomalous vessel and one or both of the normally originating coronary arteries. The two coronary arteries may arise normally from the aorta, with one or both of these vessels sending communicating branches

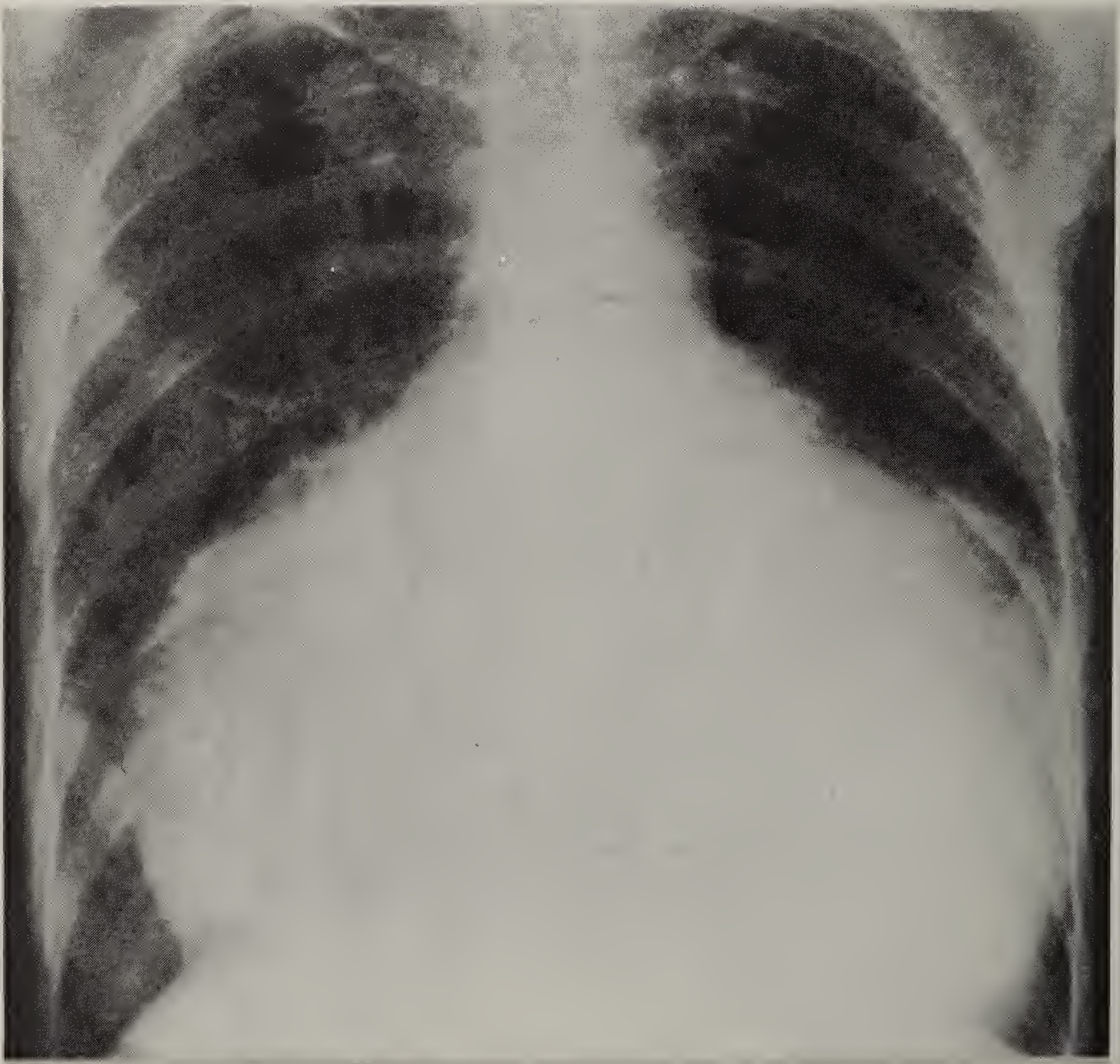

Fig. 4.—Anteroposterior view of a 54-year-old patient with a coronary arteriovenous fistula.

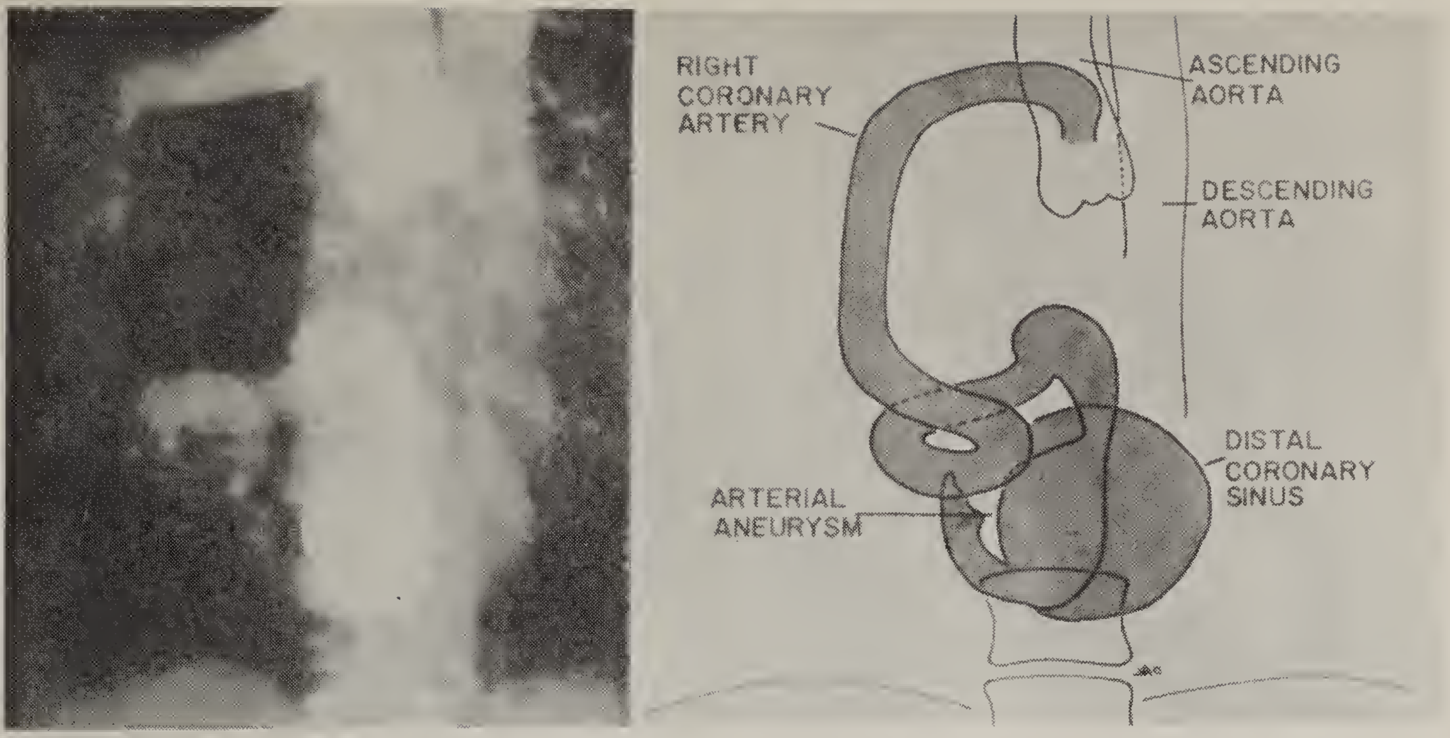

Fig. 5.—Retrograde aortogram of the patient shown in Fig. 4. The fistula involved the right coronary artery and the coronary sinus.

to a small plexus of vessels overlying the base of the pulmonary artery or the right ventricular outflow tract. Several small arteries subsequently arise from this vascular plexus to communicate with the pulmonary artery, usually above the anterior pulmonary valve. The third variant is in fact an anomalous origin of one coronary artery from the pulmonary artery, with the other coronary artery originating normally from the aorta. In this lesion the blood flow is from the aortic coronary artery through anastomoses to the pulmonic coronary artery and then into the pulmonary artery. These three types of pulmonary artery fistulas are illustrated in Figs. 6-*B* and 7-*D*.

The anomalous origin of coronary arteries from the pulmonary artery includes four variations: (1) the left coronary artery arising from the pulmonary artery, (2) the right coronary artery arising from the pulmonary artery, (3) both coronary arteries originating from the pulmonary artery, and (4) an accessory coronary artery arising from the pulmonary artery. These variations are illustrated in Fig. 7. The first listed is fairly common, the others rare.

Patients with the left coronary artery originating from the pulmonary artery are most commonly seen in early infancy with a history of feeding difficulties, frequent respiratory infections and rapid, grunting respirations. Examination reveals tachycardia, tachypnea, gross cardiomegaly, and signs of congestive heart failure. Occasionally a murmur of mitral insufficiency is present. X-rays confirm the massive cardiomegaly (Fig. 8). The electrocardiogram is usually indicative of myocardial ischemia or infarction. Retrograde aortography usually shows filling of the right coronary artery, with subsequent filling of the left coronary artery (Fig. 8) by way of intercoronary collaterals, and with the flow of contrast media then continuing into the pulmonary artery. In the infants presenting with severe myocardial ischemia or infarction, collateral flow to the left coronary artery is meager and usually inadequate for survival (Fig. 7-A). Some of the patients, however, develop an adequate collateral circulation between the right and left coronary arteries *in utero* or in early infancy, and show few or none of the characteristic signs and symptoms

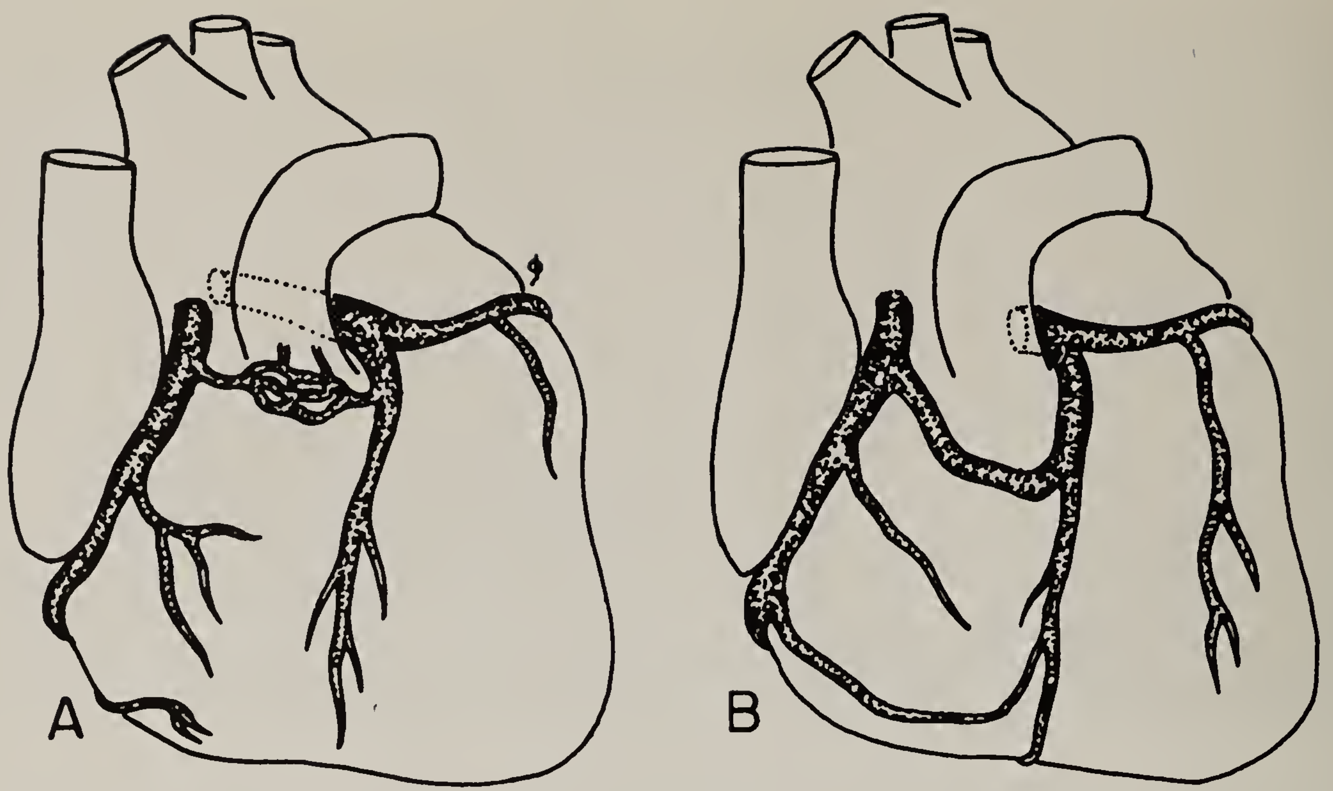

Fig. 6.—Coronary arteriovenous fistulas. A: fistula to the pulmonary artery from an arterial plexus. B: mature ("adult") stage of anomalous origin of the left coronary artery from the pulmonary artery. Compare the collateral flow with Fig. 7-A.

described above.[6] These patients may develop quite normally and, in fact, this type of lesion has been most frequently reported in adults without symptoms. Cardiac enlargement is usually present, and the electrocardiogram most frequently shows left ventricular hypertrophy. Retrograde aortography shows a large, dilated and usually tortuous right coronary artery emptying into abundant collateral vessels and then into the left coronary artery and the pulmonary artery (Fig. 6-*B*). This large flow from the aorta to the pulmonary artery behaves as an arteriovenous fistula with a characteristic continuous murmur over the upper left sternal border. Significant mitral insufficiency has been described in an occasional patient with pulmonic origin of the left coronary artery, probably secondary to papillary muscle dysfunction associated with myocardial ischemia.[7] In the infantile or immature form with inadequate intercoronary collaterals, effective surgical therapy is probably accomplished only by anastomosis from the aorta to the anomalous artery. In the adult or mature form, with adequate intercoronary collaterals, ligation of the left coronary artery at its origin from the pulmonary artery will increase myocardial blood flow by eliminating the arteriovenous fistula. It remains to be seen whether or not an aortic anastomosis is also preferable in the adult form.

Anomalous origin of the right coronary artery from the pulmonary artery (Fig. 7-*B*) is associated with no symptoms and is usually discovered at autopsy in a patient dying from an unrelated cause.

Anomalous origin of both coronary arteries from the pulmonary artery (Fig. 7-*C*) is invariably fatal immediately or within a few hours after birth. Death accompanies the closure of the ductus arteriosus and the fall in pulmonary artery pressure.

Anomalous pulmonary artery origin of an accessory coronary artery (Fig. 7-*D*) has been mentioned previously as a variety of coronary arteriovenous fistula. In this anomaly a vessel of varying size originates from the anterior pulmonary

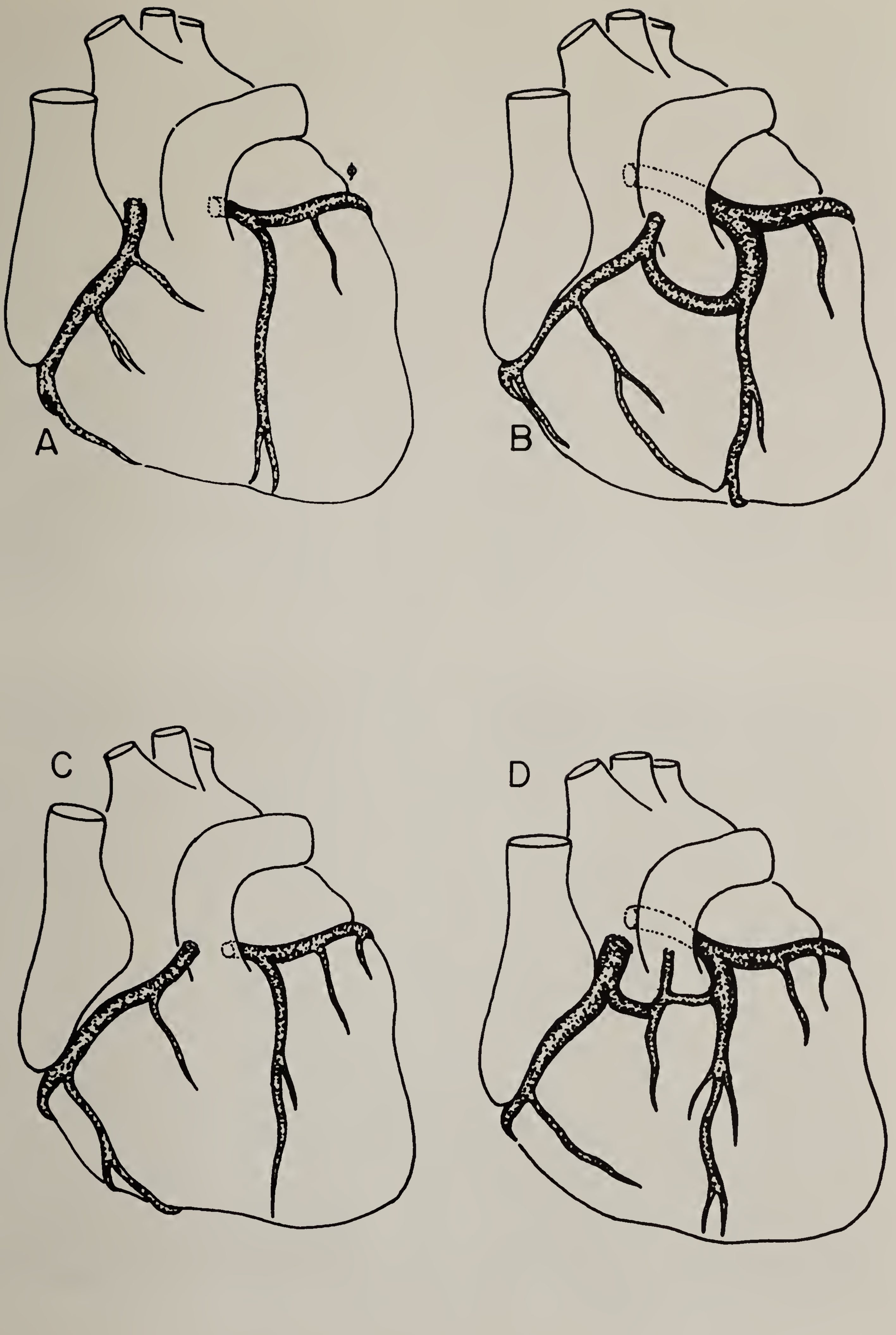

Fig. 7.—Anomalies of origin of the coronary arteries from the pulmonary artery. A: origin of the left coronary artery from the pulmonary artery; the immature ("infant") stage is shown (compare to Fig. 6-B). B: origin of the right coronary artery from the pulmonary artery. C: origin of both coronary arteries from the pulmonary artery. D: origin of an accessory coronary artery from the pulmonary artery.

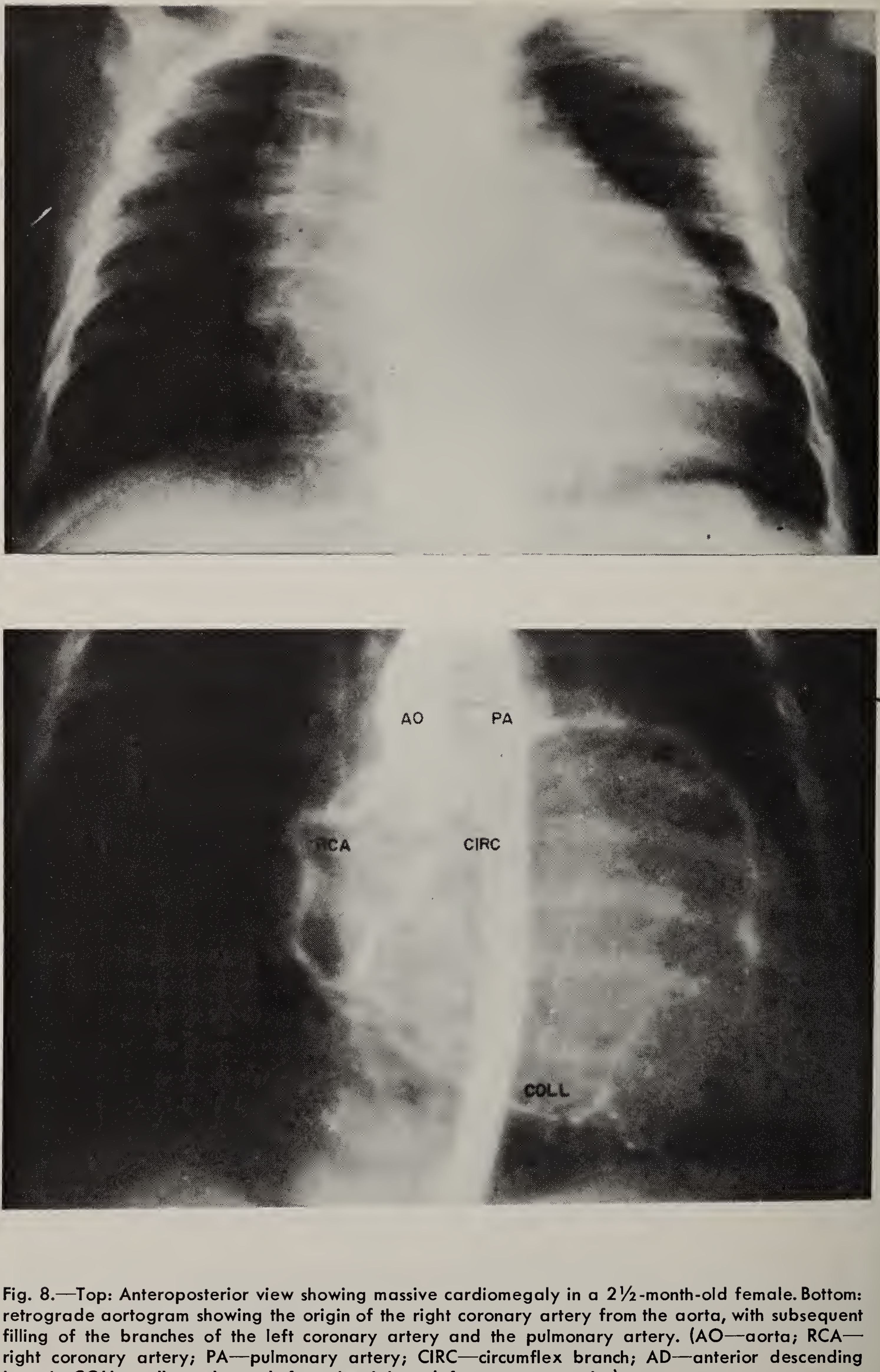

Fig. 8.—Top: Anteroposterior view showing massive cardiomegaly in a 2½-month-old female. Bottom: retrograde aortogram showing the origin of the right coronary artery from the aorta, with subsequent filling of the branches of the left coronary artery and the pulmonary artery. (AO—aorta; RCA—right coronary artery; PA—pulmonary artery; CIRC—circumflex branch; AD—anterior descending branch; COLL—collateral vessels from the right to left coronary arteries).

sinus and descends over the right ventricular outflow tract. Most are small. They are connected to one or both of the normally originating coronary arteries, and thus the accessory artery serves as a connection between the high pressure coronary arterial system and the low pressure pulmonary artery.

A single coronary artery is an infrequently reported lesion usually seen at autopsy, although awareness of its potential presence makes an antemortem diagnosis possible.[8] This anomaly has also been referred to as an absent coronary artery. Smith[9] has described three variations: (1) a single coronary artery with the basic distribution of only one of the coronary arteries, (2) a single coronary artery which immediately branches to follow the distribution patterns of both coronary arteries, and (3) a single coronary artery without any normal pattern of distribution. However, these variations result only because one set of intercoronary fetal anastomoses develops in preference to another. Thus, in the first variation, the anastomoses between the right coronary artery and the left circumflex branch on the posterior surface of the heart open (Fig. 9-*A*), whereas in the second variation, anastomoses around the great vessels are the preferential channels (Fig. 9-*B*).

A similar anomaly is that which comes about when there is absence of a coronary artery, as in the single coronary artery, but two or three coronary vessels originate from the other coronary sinus (Fig. 10).

Another situation similar to the single coronary artery is that of a hypoplastic coronary artery (Fig. 11). In the example illustrated, the right coronary artery was hypoplastic, extending for only two centimeters from the aortic origin. The artery present in the right atrioventricular groove was a continuation of the circumflex branch of the left coronary artery.

Minhas has described an infant with

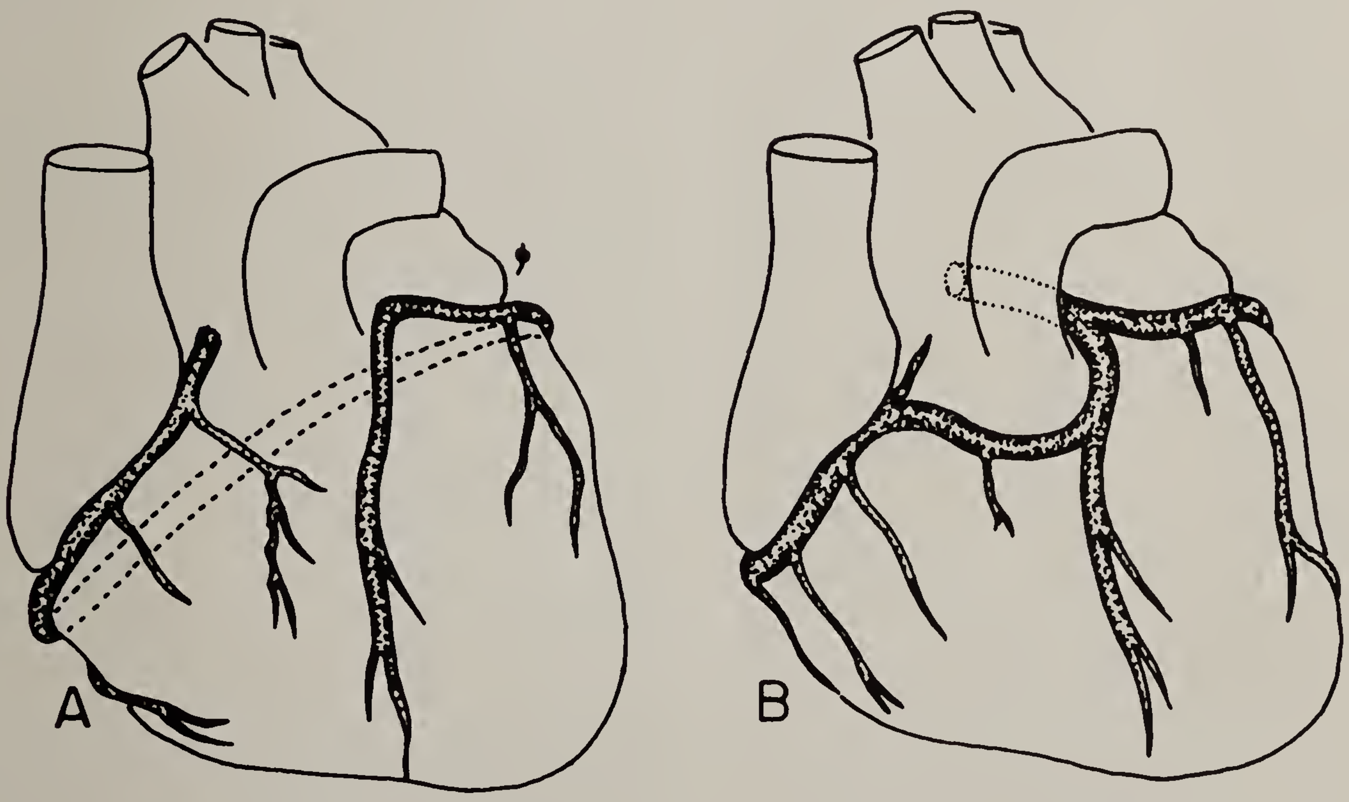

Fig. 9.—Single coronary artery. A: single right coronary artery—Smith Type 1. B: single left coronary artery—Smith Type 2.

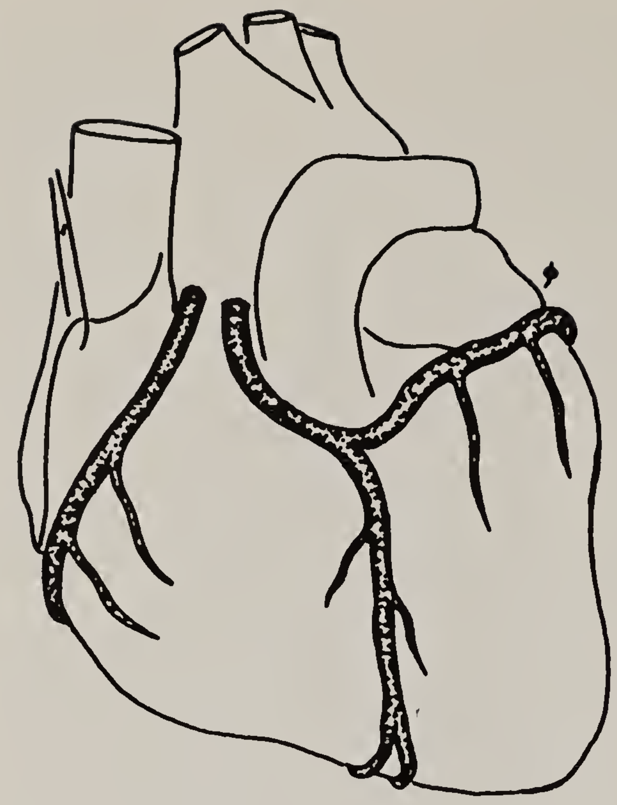

Fig. 10.—Atresia of the ostium in the left aortic sinus with origin of both arteries from the right aortic sinus.

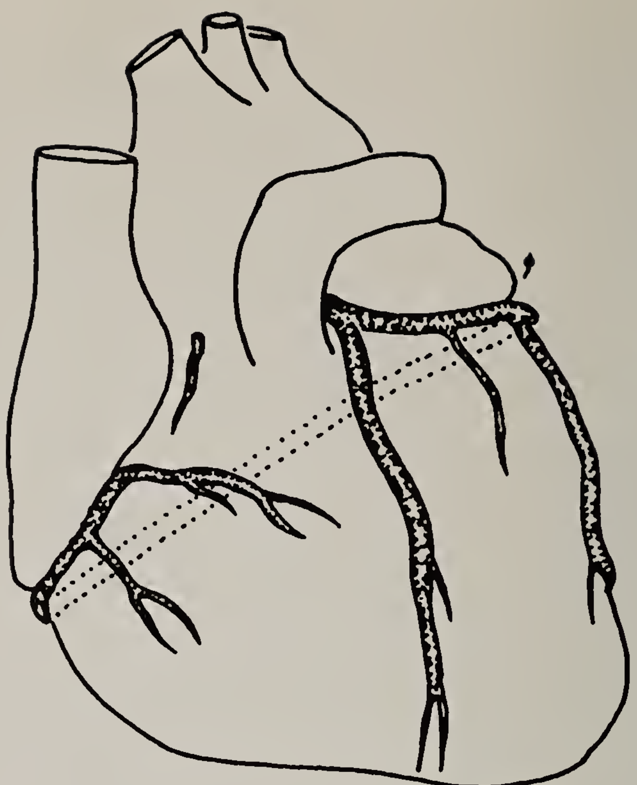

Fig. 11.—Hypoplasia of the right coronary artery with continuation of the circumflex branch along the right atrio-ventricular groove.

hypoplasia of the anterior descending coronary artery.[10] Despite an otherwise normal coronary artery distribution, the child had signs and symptoms suggestive of anomalous origin of the left coronary artery from the pulmonary artery, including electrocardiographic signs of an anterolateral myocardial infarction. The lesion was demonstrated by coronary arteriography.

An isolated hypoplastic segment of a coronary artery has been reported by Smol'innikov and Naddachina.[11] A similar example was found in this case review, involving the right coronary artery (Fig. 12). About one centimeter from the aortic origin the right coronary artery became hypoplastic for two centimeters, at which point it again assumed a normal diameter and continued its course in the right atrioventricular groove, terminating as the posterior descending artery. There were no grossly patent collaterals from the left circumflex branch, although the left coronary artery was slightly larger than normal.

The Secondary Coronary Artery Anomalies:

The lesions in this group are found in association with additional congenital malformations of the heart and are considered to be compensatory or adaptive developmental aberrations. Fig. 13 depicts such an anomaly in which communications have been established between the right ventricle and the coronary arterial system in the presence of pulmonary valve atresia or severe stenosis, intact ventricular septum, hypoplasia of the right ventricle, and tricuspid stenosis. Similar coronary-left ventricular communications may occur in the presence of aortic valve atresia or severe stenosis with hypoplasia of the left ventricle and mitral valve. The coronary communications in these instances serve as means of egress of blood from the otherwise completely or severely obstructed ventricles.

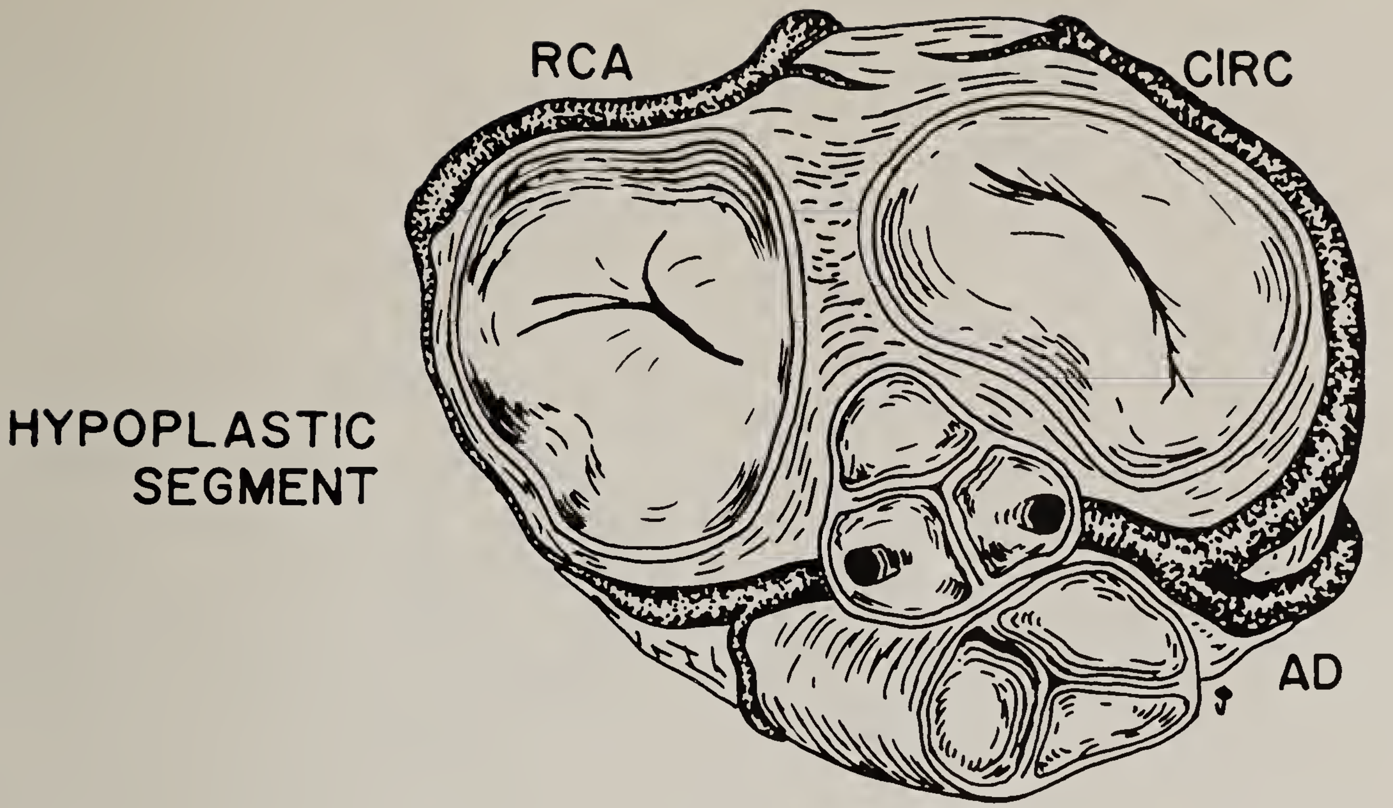

Fig. 12.—Hypoplasia of a segment of the right coronary artery with isolation of the distal portion of the right coronary artery.

Anomalies of the coronary arteries seen in transposition of the great vessels involve both the origin and distribution of the arteries. Two of these are illustrated in Fig. 14. The frequent variations encountered in association with this complex are basically a result of malposition and rotation of the aortic root, with a vessel emerging as the right coronary artery proceeding to the distribution of the branches of the left, and conversely the distal branches of the right coronary artery originating from an anatomical left coronary artery. Anomalies of origin of the coronary arteries are also encountered, although not as frequently as discrepancies of the distribution.

Anomalous coronary artery branches are not infrequently encountered at the site of right ventriculotomy in the repair of tetralogy of Fallot. Failure to recognize and avoid or ligate one such branch was undoubtedly responsible for the development of a coronary artery-right ventricular fistula at the site of a ventriculotomy.[12]

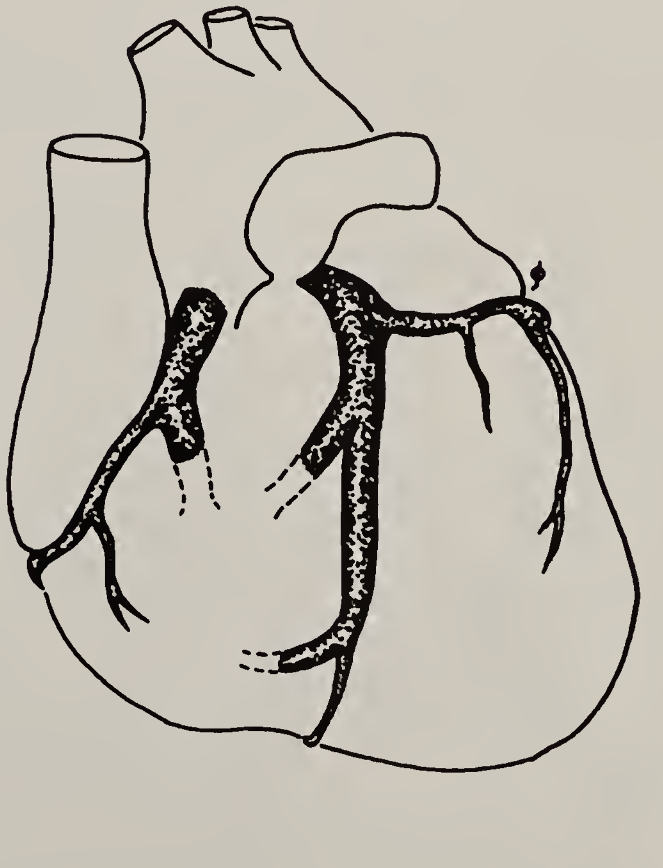

Fig. 13.—Secondary coronary arteriovenous fistulas arising in a heart with atresia of the pulmonary valve and a patent ductus arteriosus.

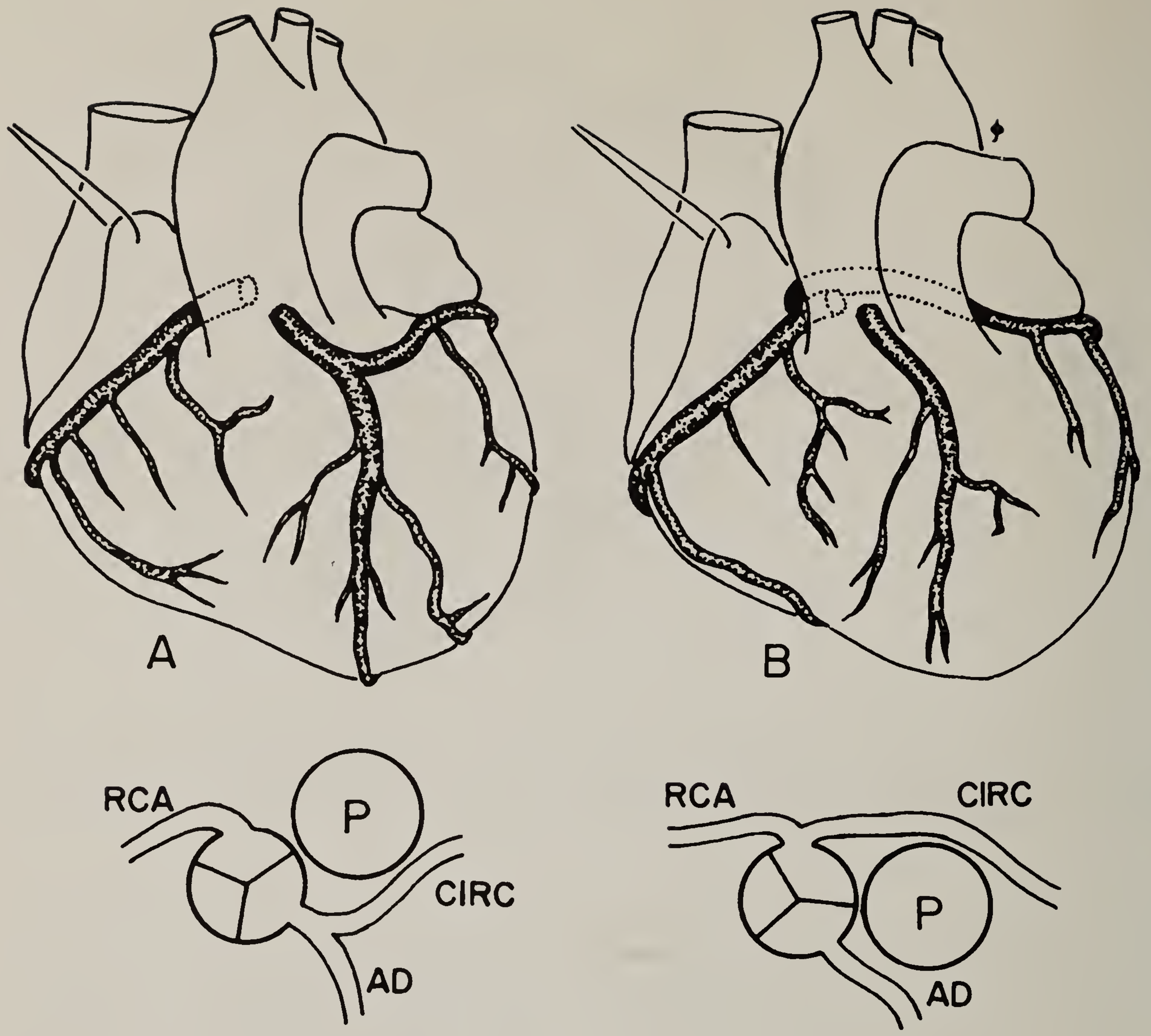

Fig. 14.—Transposition of the great vessels. A: predominant circulatory pattern in the anteroposterior relationship. B: predominant pattern in side-to-side relationship.

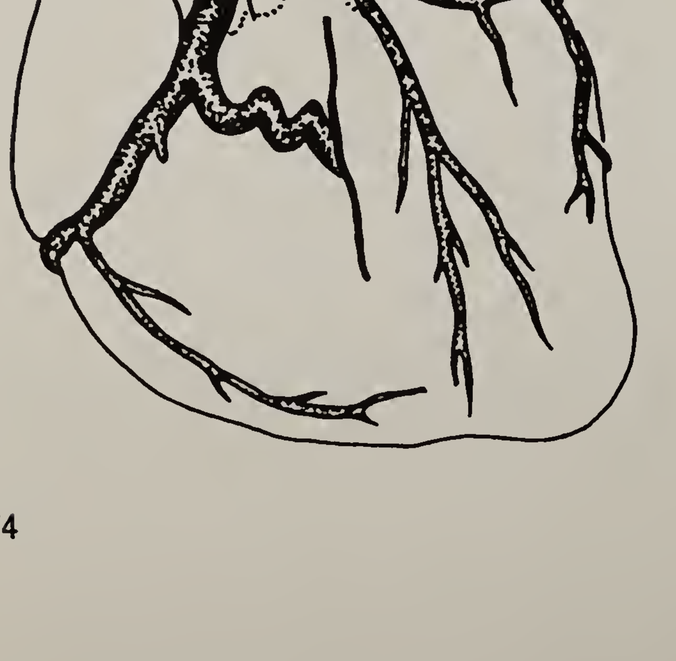

Fig. 15.—Coronary arteriovenous fistula from the right coronary artery to the right ventricle. This fistula arose following ventriculotomy.

The lesion became apparent with the development of a continuous murmur several months postoperatively. The fistula was demonstrated by retrograde aortography and was closed surgically. A specific coronary artery anomaly was not recognized at either operation.

SUMMARY

A discussion of the anomalies of the coronary arteries is presented, based upon a review of the records of 182 patients with these lesions documented clinically, by appropriate laboratory procedures, at operation, and at autopsy.

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ABSTRACTS

OF PUBLICATION BY THE STAFF

Booyse, F. M. and Rafelson, M. E.: Studies on Human Platelets. I. Synthesis of Platelet Protein in a Cell-Free System. *Biochim. Biophys. Acta, 166:689-697, 1968.*

A reproducible cell-free system, active in protein synthesis has been prepared from human blood platelets. The ability of this system to incorporate ^{14}C-labeled amino acids, is dependent on the presence of ribosomes, a soluble enzyme fraction (S-150), and ATP *plus* an ATP-generating system. Both puromycin and ribonuclease have the ability to inhibit amino acid incorporation. A major soluble protein product of this system has been characterized; the solubility properties, sucrose gradient patterns, and fingerprint patterns of the ^{14}C-labeled soluble product appear identical to the [^{14}C]actomyosin-like contractile protein isolated from labeled, intact platelets.

Booyse, F. M. and Rafelson, Max E. Jr.: Hypothesis: Studies on Human Platelets III. A Contractile Protein Model for Platelet Aggregation. *Blood, 33:100-103, Jan. 1969.*

A new hypothesis is proposed for platelet aggregation. The model involves the formation of *intermolecular* platelet actomyosin bridges between platelet actin and platelet myosin moieties localized on or near the surface of adjacent platelets. These *intermolecular* platelet actomyosin bridges can then contract in the presence of ATP, drawing the platelets into a tight mass or thrombus.

Bezkorovainy, Anatoly, Grohlich, Dietmar, and Gerbeck, Claire M.: Some Physical-Chemical Properties of Reduced-Alkylated and Sulphitolysed Human Serum Transferrins and Hen's-Egg Conalbumin. *Biochem. J., 110:765-770, 1968.*

1. Apparently all disulphide bridges of transferrin and conalbumin were broken by reduction-alkylation, whereas sulphitolysis resulted in incomplete cleavage of disulphide bonds. 2. The molecular weights of reduced-alkylated and sulphitolysed transferrin and reduced-alkylated conalbumin were identical with those of native proteins in a number of solvents, indicating that these proteins exist as single polypeptide chains. 3. Viscosity studies indicated that reduced-alkylated transferrin possesses a partially ordered structure in 0-4M-urea, assumes a random-coil configuration in 6M-urea with a molecular weight of 84000 and is partially aggregated in 8M-urea.

Hartman, J. Francis: Newer Knowledge of the Neuron. In: *The Central Nervous System, International Academy of Pathology Monograph Series,* **O. T. Bailey and D. E. Smith, eds. Baltimore, The Williams & Wilkens Co.,** *1968. Chapt. 1.*

Although electron microscopy has revealed little in the way of ultrastructure of nerve tissue that is sufficiently unique as to explain at one stroke its highly developed conductive properties, the current views regarding normal neuronal fine structure can be presented as a groundwork for neuropathologic studies in which the electron microscope has been used. The neuronal nucleus is ultrastructurally similar to the interphase nucleus of other cell types, with a two-layered nuclear envelope exhibiting nuclear pores, and with the outer of the two layers in continuity with the endoplasmic reticulum. The cytoplasm contains the usual complement of subcellular structures, such as endoplasmic reticulum. Golgi material, mitochondria, lysosomes, etc., the structure and arrangements of which are described and illustrated. Special features of neuronal processes, i.e., of dendrites, myelinated axons, unmyelinated axons and synaptic terminals are discussed, and shown in representative electron micrographs.

Sabet, Tawfik Y. and Ray, Robert D.: Failure of Ribonuclease to Inhibit the Transfer of Homograft Sensitivity. *Clin. Orthop., 61:92-102, Nov.-Dec. 1968.*

The effect of RNase treatment on the transfer of allograft sensitivity has been studied. Lymphoid cells from CBA mice previously sensitized to A strain mouse cells were incubated either with RNase or PBS and transferred to isologous CBA hosts. Five days later the hosts were challenged with A strain skin and bone allografts. The skin grafts were recovered after 7 days and bone after 10 days for histologic evaluation. The results indicate that skin is a more sensitive tissue for detection of accelerated rejection. Successful transfer of sensitivity was obtained in all the mice which received lymphoid cells incubated in PBS and in 92% of the mice which received cells incubated in RNase. The results indicate that RNase does not inhibit transfer of homograft sensitivity.

Kuettner, Klaus E., Guenther, Harald L., and Ray, Robert D.: Lysozyme in Preosseous Cartilage. *Calc. Tiss. Res., 1:298-305, 1968.*

A lytic substance was found in calf scapular cartilage and identified as a lysozyme by the following criteria: It lysed the specific substrate for lysozyme, *Micrococcus lysodeikticus;* it was stable at low pH and temperatures up to 100°; it was unstable above pH 7; and it behaved as a cation identical to chicken egg white lysozyme electrophoretically at pH 8.4. The lysozyme was extracted quantitatively from calf nasal septa and from the zones of transformation of calf scapular cartilage and new bone. There were indications that the cartilage lysozyme was at least in part extracellular and bound to the mucoprotein. Resting cartilage of calf scapula was found to contain more lysozyme than that of nasal septa. A significant rise in lysozyme concentration was found toward the ossification front in scapular cartilage as was a sharp drop in new bone. Studies on fresh ossifying scapular cartilage of dogs of different ages indicated a decrease in lysozyme concentration as the animal matured and the growth rate of bone decreased.

Schrodt, Merle, J., Eisenstein, Rueben, Ray, Robert D., and Kuettner, Klaus E.: Lysozyme in Embryonic Cartilage: Ontogenic Studies. *Surg. Forum, 19:461-462, 1968.*

The more mature tissue culture specimens with bone already present were the only ones to show a definite histologic response to lysozyme. The quantitative studies reflect the fact that lysozyme activity parallels the development of the bone. These data also show that lysozyme is present in avian cartilage and that it induces similar histologic changes to those previously reported in dog, calf, and human cartilage. It has thus been shown that when lysozyme is obtained from the same species as the cartilage, it induces changes similar to those induced in other species. The result described here cannot, therefore, be attributed to the action of lysozyme as a foreign protein. This strengthens the idea that lysozyme has a physiologic role in cartilage and bone.

Kuettner, Klaus E., Ray, Robert D., and Yaeger, James A.: Lysozyme and Preosseous Cartilage. *J. Bone Joint Surg., 50:1072, July, 1968.* (Abstract).

A lytic substance was found in calf scapular cartilage and identified as lysozyme. It was extracted quantitatively from nasal septa and from the zones of transformation of scapular cartilage and new bone. The cartilage lysozyme was at least in part extracellular and bound to the mucoprotein. Resting scapular cartilage was found to contain more lysozyme than nasal septa. A significant rise in lysozyme concentration was found toward the ossification front in scapular cartilage. In bone, the concentration was low. There was a decrease in lysozyme concentration as the animal matured. Tissue culture of preosseous cartilage showed an increase in opacity not due to calcification when lysozyme or protamine was added to the culture media. The cartilage matrix became more eosinophilic and P.A.S.-positive.

Kuettner, K. E., Soble, L. W., Eisenstein, R., and Yaeger, J. A.: The Influence of Lysozyme on the Appearance of Epiphyseal Cartilage in Organ Culture. *Calc. Tiss. Res., 2:93-105, 1968.*

1. A new method is described for preparing the osteocartilaginous junction of growing dog scapular cartilage free of periosteum. This preparation is suitable for organ culture.
2. Addition of lysozyme to organ culture of such material results in an increase in opacity of the tissue. This "darkening" is induced in viable epiphyseal cartilage, but not in non-viable epiphyseal cartilage or in cartilage which does not ossify (nasal septum).
3. Protamine, another basic protein, also induces "darkening" which, in contrast to lysozyme, is persistent after withdrawal of the protein.
4. The "darkening" induced by lysozyme, but not protamine, spares the zone of hypertrophic cartilage cells, the zone where lysozyme is present in highest concentration *in vivo*.
5. Histologically, both lysozyme and protamine induce changes in the staining reactions of the cartilage matrix. The distribution of these changes appears to be the same as that of the gross darkening effect.
6. Microradiography indicates that these changes are not a consequence of calcification.
7. These data support the concept that lysozyme may have a functional role in either osteogenesis, chondrogenesis, or calcification.

Apter, Julia T. and Marquez, Elsa: A Relation Between Hysteresis and Other Viscoelastic Properties of Some Biomaterials. *Biorheology, 5:285-301, 1968.*

The behavior of linear viscoelastic materials may be approximated by differential equation of motion whose solution makes it possible to establish a relation between viscoelastic parameters obtained from stress relaxation studies and those obtained from oscillatory studies. Living tissues behave like linear viscoelastic materials under some conditions so that parameters may be determined from the two kinds of studies and compared with each other to establish the standard linear solid as a model. This study succeeds in showing the model to be suitable under a limited range of conditions for a variety of viable tissues tested *in vitro.*

Chow, Joseph C. F., and Apter, Julia T.: Wave Propagation in a Viscous Incompressible Fluid Contained in Flexible Viscoelastic Tubes. *J. Acoust. Soc. Amer., 44:437-443, Aug. 1968.*

The dispersion and attenuation of waves in viscoelastic tubes filled with a viscous incompressible fluid are analyzed, with special attention to two cases: where the constitutive relation of the wall is that (1) of a Voigt material, and (2) of a standard linear solid. Explicit expressions show the dependence of the phase-velocity and damping factor on the various parameters characterizing the fluid and the viscoelastic tube. In comparison with elastic tubes, viscoelastic tubes have a higher phase velocity and attenuation coefficient. Also, as the ratio of the relaxation time of the viscoelastic material to the period of the oscillation increases, there is a marked difference between the behavior of the waves for the two types of materials considered. The frequency equation for a stretched elastic tube is also derived. The analysis is restricted to waves whose amplitude is infinitesimal with the wavelength large and the wall thickness small as compared with the radius of the tube.

Apter, Julia T. and Najafi, Hassan: A Test for Transplantability of Animal Hearts. *J.A.M.A. 206:2881, Dec. 1968.*

This study used several methods widely applied in the measurement of the viscoelastic properties of artificial materials and of other biological tissues but apparently not previously used on cardiac muscle. These methods include measurement of the absolute dynamic extension modulus and of phase shift from stress-strain loops taken over a wide range of frequencies along with the thermal response of the modulus. The finding that the dynamic modulus increased with time as rigor mortis developed and that the time course was faster at 37 C than at 0 C is in keeping with the reports on extensibility by Griggs *et al.* and by Kolder *et al.* The finding that rigor mortis developed much more slowly in Ca^{++}-free solutions is in keeping with the report by Weiner and Pearson on rabbits treated with ethylenediaminetetraacetic acid (EDTA). It has not previously been reported that the temperature-modulus relationship remains negative and reversible only so long as ATP levels are normal. This is in keeping with a prediction by Szent-Gyorgyi.

What seems clear are (1) that the thermal response of the viscoelastic properties of the atrial appendage is a reliable indicator of the response of other portions of the heart; (2) that fresh cardiac muscle is in a steady state with regard to the thermal response of its elastic modulus and the phase shift; and (3) that muscle developing rigor mortis is in a transient state easily diagnosed by transient changes in viscoelasticity tested by accepted methods and confirmed by disappearance of ATP.

Apter, Julia T. and Marquez, Elsa: Correlation of Viscoelastic Properties of Large Arteries with Microscopic Structure. V. Effects of Sinusoidal Forcings at Low and at Resonance Frequencies. *Circ. Res. 22:393–404, Mar. 1968.*

Nine aortas from recently killed dogs were sectioned into 21 or more ring segments supported horizontally on two hooks in Ringer's solution. One hook oscillated sinusoidally from .01 to 21 Hz to stretch the segments 1.2% in excess of 4 or more mean strain levels from 5 to 100%. The segments were kept at 4 temperature levels (0°, 20°, 37°, 60° C). The other hook was coupled to a force transducer. At frequencies below 1 Hz, the force registered was sinusoidal with the same frequency as the stretch which it led, unless the specimen contained demonstrably contracted smooth muscle; then the stress was nonlinear and lagged behind the strain at frequencies below .05 Hz. As frequencies rose above 1 Hz, the force amplitude rose to a maximum, resonating at frequency ω_r, which was higher at higher initial strains. Concurrently the phase shift increased to 90° at another frequency ω_{90}. When $\omega_{90} = \omega_r$, viscous losses were negligible, a fact confirmed by other data in this and other studies and generally implicating collagen. When $\omega_{90} > \omega_r$, viscous losses were appreciable, in agreement with other measurements and implicating either muscle or elastin. These two wall components could be distinguished from each other by responses to drugs and to changes in temperature. Absolute, dynamic modulus, storage modulus, loss modulus, phase shift, and loss angle measured from stress-strain loops compared favorably with similar measurements published for other viscoelastic materials and with viscous and elastic constants obtained from stress-relaxation experiments on aorta.

Gubisch, N. J., Wolter, J., Perlia, C. P., Dederick, M., Ream, N. W., and Taylor, S. G., III: The Use of Mithramycin in Malignancy-Associated Hypercalcemia. *Proc. Am. Assoc. Cancer Res., 9:26, 1968.*

The frequent association of hypocalcemia with the administration of Mithramycin, which appeared unrelated to antitumor activity or site and degree of metastatic involvement, prompted its trial in the control of hypercalcemia. It was found to be effective in a dose range well below that which usually produces toxicity.

If the clinical situation permitted, baseline studies were obtained on forced hydration alone. Mithramycin was then given as a single bolus injection of 5-20 μg/kg. The optimal dose was directly related to, and therefore, guided by, the degree of calcium elevation. In those patients where dose-related nausea precluded the bolus injection, a 24-hour infusion was used. Calcium levels approached normal within 24 hours and remained so for an average of four days. A rapid drop to hypocalcemic levels appeared to shorten the period of effectiveness suggesting triggered release of thyrocalcitonin.

Urine calcium excretion reflected serum calcium levels during administration of the drug. No difference was noted in those patients maintained solely on calcium-free intravenous fluids, making decreased G.I. absorption of calcium an unlikely mechanism of action; however, increased secretion of calcium into the gut was not ruled out. The inhibition of bone resorption as the primary site of action, suggested in animal studies, remains to be demonstrated in man.

Northrop, Gretajo, Taylor, Samuel G., and Northrop, Robert L.: Mithramycin-Induced Inhibition of RNA Synthesis and its Reversibility in BHK-21 Cells. *Proc. Am. Assoc. Cancer Res., 9:55, March 1968.*

Mithramycin (M), an antibiotic used primarily in the treatment of testicular and neurological tumors has been found to inhibit incorporation of p^{32} into RNA. DNA viruses were inhibited by M in tissue culture; RNA viruses were not. Removal of M from the culture permitted DNA viral growth to continue. Results to be presented show that M specifically inhibits cellular RNA synthesis and that the inhibition is reversible. BHK-21 cells were treated 4 hrs. at 37° C with M (0.1–400 μgm/ml), then labeled with uridine-H^3 (5 μc/ml) for 1 hr. The TCA insoluble cellular extract was then counted. Data obtained showed as much as 85% inhibition of RNA synthesis. This inhibition of RNA synthesis is dose-related in M concentrations between 1–400 μgm/m. Removal of M from the media of BHK-21 cells showing 70% inhibition of RNA synthesis was followed by reversal of inhibition and return of the rate of RNA synthesis to that of the untreated controls. Comparison of cytoplasmic components of M (20 μgm/ml) treated and untreated BHK-21 cell homogenates, separated on sucrose density gradients showed a marked decrease of uridine-H^3 incorporation into all classes of RNA. M(1–400 μgm/ml) also partly inhibited, unrelated to dose, amino amino acid-C^{14} and thymidine-H^3 incorporation into TCA insoluble extracts.

Schwartz, F. D.: Prepared Statement On The Problem Of Urine Enzyme Determinations. In *Enzymes in Urine and Kidney*, **ed. H. Albi, H. Mattenheimer and F. W. Schmidt. Bern and Stuttgart, Hans Huber,** *1968, p. 296.*

Dialyzable inhibitors were demonstrated in urine against LDH and alkaline phosphatase, but not for MDH, LAP, GOT or amylase. The methods used achieved "recovery" of 70–110% for homogenate added to urine. Alternations of pH, NaCl and urea concentrations within the physiologic range did not alter urinary enzyme activity. Gross genitourinary tract bleeding should rarely influence urine enzyme determinations.

In neither animal nor human studies was a significant renal extraction of serum enzymes demonstrated with the exception of amylase. This is probably due to limitation of glomerular permeability. While molecular weight of amylase is only 40,000, the remainder are 110,000 or greater with the exception of MDH. Of course in disease states associated with increased glomerular permeability, i.e., the nephrotic syndrome, some extraction of serum enzyme might be expected. This is presently under investigation.

On the other hand, under certain conditions release of renal enzyme can make a significant contribution to serum enzyme activity. The primary source of urinary activity would appear to be breakdown and release by renal tubular epithelium and any increase in urinary enzyme activity merely reflects a non-specific effect of renal injury.

Mattenheimer, Hermann: Enzymology of Kidney Tissue. In *Enzymes in Urine and Kidney*, **ed. H. Albi, H. Mattenheimer and F. W. Schmidt. Bern and Stuttgart, Hans Huber,** *1968, pp. 119-145.*

The origin of enzymes in urine has recently been discussed in detail and the following conclusion was reached. Renal tissue must be regarded as the main source for enzymes in urine. In the healthy human kidney only enzymes with a molecular weight of less than about 70,000 can be filtered in the glomeruli. By this mechanism amylase (molecular weight 45,000), an enzyme not present in kidney tissue, gets into the urine. Of the enzymes which have been more thoroughly investigated in urine and which are present in both, renal tissue and serum (LDH, MDH, AP, LAP), only MDH (molecular weight 40,000–60,000) can be assumed to be filtered. Probably not all of the filtered enzyme is excreted with the urine, because proteins are reabsorbed in the proximal tubuli. The greater portion of MDH in urine therefore most likely is derived from kidney tissue.

The LDH-isoenzyme pattern in normal human urine with predominantly LDH-1 is consistent with the conception that enzymes in the urine are derived mainly from the cortex.

Whether or not serum enzymes can penetrate the glomerular epithelium in renal disease is yet unknown. Most authors did not find any correlation between proteinuria and enzymuria. One has to assume therefore that the larger portion of enzymes in urine of patients with kidney disease originate from tubular cells.

Hendry, Derek P., and Dillow, P. V.: Observing Behavior During Interval Schedules. *J. Exp. Anal. Behav., 9:337-349, July, 1966.*

Experiment 1 showed that the three stimuli associated with three chained fixed-interval links could be used to maintain observing behavior. Experiment 2 showed that three stimuli correlated with the passage of time since the last reinforcement in a fixed-interval schedule could be used to maintain observing behavior. In both experiments most observing responses occurred midway between reinforcements. Few occurred just before or just after reinforcement. Experiment 3 showed that the decline in the rate of observing behavior just before reinforcement was reduced when more stimuli could be observed. The relatively high terminal rate of observing behavior that resulted was maintained even when at least 4 sec intervened between the reinforcement and the last observed stimulus.

Hendry, Derek P., and Van-Troller, C.: Alleviation of Conditioned Suppression. *J. Comp. Physiol. Psychol., 59:458-460, 1965.*

During 39 sessions of conditioned suppression training, the US intensity for 6 rats was 2 mA and for 6 others 1 mA for 24 sessions, then 2 mA for 15 sessions. Initial suppression was followed by recovery of responding during CS and intervening control periods. Control response rate was not affected by US intensity, but rate during CS was. The less severe suppression with 1-mA US was maintained after US intensity was increased to 2 mA. Suppression ratios showed faster recovery over the 1st 24 sessions with 1-mA US and the higher level of recovery was retained under 2-mA US intensity. With 1-mA US an acceleration of response rate followed CS onset.

Hendry, Derek P., Yarczower, Matthew, and Switalski, Richard C.: Periodic Shock with Added Clock. *J. Exp. Anal. Behav., 12:159-166, Jan. 1969.*

Rats were shocked every 6 min. while responding was maintained on a variable-interval schedule of reinforcement. With some rats, shocks were interspersed with a sequence of three different stimulus conditions (S3→S2→S1), or clock cues, each lasting 2 min. For other rats, a single stimulus condition prevailed between shocks at the beginning of the experiment and clock cues were introduced later. Response rate decreased from S3 to S1. Response rate in S, S2, and S1 was inversely related to shock intensity. When clock cues were added, response rate increased in all 2-min. intershock periods. During clock cues, an index of curvature, indicating the degree of negative acceleration of response rate, was greatest for S1 and least for S3, and was directly related to shock intensity. The response-facilitating effect of shock and its relation to a possible discriminative function of shock and to behavioral contrast is discussed.

Millburn, Lowell F., O'Grady, Lois, and Hendrickson, Frank R.: Radical Radiation Therapy and Total Body Irradiation in the Treatment of Ewing's Sarcoma. *Cancer, 22:919-925, Nov. 1968.*

The results presented in the recent literature on the treatment of Ewing's sarcoma are more than encouraging. Radiation therapy has been and remains the treatment of choice in this disease. The concept of irradiating the entire bone of involvement with high doses in short periods of time has only recently been reached. The misinterpreted clinical observation that the rapid response of this tumor to ionizing radiation meant that it could be controlled by low doses has been slow to die. Many authors have advocated including the entire bone in the irradiated volume, yet no reported series states that this was done to all patients treated. In this article the authors present their experience with total body radiation and Ewing's sarcoma in addition to the historical development of this treatment. Particular emphasis is placed on the radiotherapeutic approach to bone tumors and the feasibility of managing patients undergoing total body radiation of this magnitude.

Hendrickson, Frank R., and Liebner, Edwin: Results of Preoperative Radiotherapy for Supraglottic Larynx Cancer. *Ann. Otol., 77:222, 1968.*

A study has been presented to compare the effects of low dose with high dose pre-operative radiation therapy on a well-defined group of advanced supraglottic cancers. We have been unable to show a superiority of one dose over the other. The surgery has been performed without a mortality and with only a minor increase in morbidity. A retrospective comparison with patients in the same hospitals treated in the preceding two years suggests improvement of results with the current program. Our present conclusions would be to strongly recommend a low dose, short course, prompt surgery program for operable, locally advanced epidermoid carcinoma where local persistence is a major factor in failure. Higher dose preoperative programs may be of value in inoperable lesions. A prospective random study of surgery only, versus low dose pre-operative treatment, is desperately needed to remove the problems of retrospective comparison.

Traut, Eugene F.: Placental Extract Effective in Arthritis. *Illinois. Med. J., 135:75, Jan. 1968.*

Recently delivered placentas yield an aqueous extract capable of frequently and markedly ameliorating the symptoms and findings of some common types of joint disease.

Traut, Eugene F.: Fibrositis, *J. Amer. Geriat. Soc., 16:531, 1968.*

Fibrositis—muscular or nonarticular rheumatism—is a controversial diagnosis; nevertheless it represents a real entity, whether primary or secondary. The outstanding symptoms are pain and stiffness. Objective findings are "trigger areas" and indurations. The most common sites are the soft tissues of the neck, shoulder, elbow, carpal tunnel, palms (Dupuytren's contracture) and low back, but sometimes the syndrome is generalized.

Therapy for each type is outlined. The immediate treatment usually includes the administration of analgesics, immobilization of the affected area, and infiltration of the trigger points with procaine and adrenocortical steroids. Physical therapy (heat, massage and described exercises) is indispensable.

Ben-Porath, Edna, and Northrop, Robert L.: Synthesis of Actinomycin D-insensitive RNA in Persistent Infection with Mumps Virus. *Fed. Proc., 27:615, 1968.*

CM cells are Chang's human conjunctiva (CHC) cells persistently infected with mumps virus. Viral antigens accumulate in the growing cells but little, or no virus is released. Active virus excretion occurs when cell growth is reduced. For the present studies, cultures were labeled with H^3-uridine (URH^3) in the presence or absence of actinomycin D (AD). Incorporation of URH^3 into cellular components was analyzed in sucrose density gradients. In *growing* CM and CHC cells 90% of the label was associated with heavy membrane fraction. After treating the homogenates of either cells with deoxycholate (DOC) the radio-activity was recovered mostly in the preribosomal components. AD inhibited 90% of the RNA synthesis. In *non growing* CM and CHC cells the labeled RNA was again found in heavy membrane fraction. However, after treating with DOC a labeled heavy component was seen in CM cells which did not appear in CHC cells. This newly synthesized RNA synthesis could only be demonstrated in the nongrowing cells under these conditions.

Deinhardt, Friedrich and Holmes, Albert W.: Transmission of Human Viral Hepatitis to Nonhuman Primates. *Perspect. Virology, 6:89-103, 1968.*

1. It has been shown that marmosets develop a hepatitis which is comparable in almost every respect to the human disease and which can be transmitted from animal to animal with regularity by not just one but by any laboratory using the same species of animals.
2. No known agent appears to be responsible for this disease.
3. No neutralizing antibodies have been demonstrated, either in human or in marmoset convalescent serums, but resistance to a second challenge infection develops in marmosets infected with either one of the two agents (WW-55 and GB) which we have studied most extensively.
4. The physical and chemical characteristics of the agent responsible for the disease are unknown.
5. There is an indication that the causative agent of this hepatitis can be maintained in marmoset lung cell cultures for 8 cell passages—about 10 weeks—but this is preliminary evidence and must be supported by considerably more work and be confirmed by other laboratories.
6. We have no certain proof that the disease observed in marmosets is caused by "the" or "one" of the agents of human viral hepatitis. The possibility of an accidental pickup of a marmoset agent exists but does not seem too likely.

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Antibodies to Epstein-Barr Virus

Evaluation of Pituitary Function by Growth Hormone Response

Computers in Psychiatry

Abstracts

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DISTRIBUTION OF ANTIBODIES TO EPSTEIN-BARR VIRUS IN VARIOUS AMERICAN POPULATION GROUPS

Grace J. Shramek
Friedrich Deinhardt
Peter Tischendorf
Rolando C. Balagtas
William H. Knospe
James E. Maynard
Gary R. Noble

The herpes-like Epstein-Barr virus, or EBV, which was originally isolated from Burkitt's lymphoma[1] was first incriminated as the cause of infectious mononucleosis by G. and W. Henle and their co-workers who demonstrated that antibodies to EBV rose during the disease[2,3] and also isolated EBV from acute phase lymphocytes.[4] Evans[5] has postulated that infectious mononucleosis is a frequent but usually unrecognized infection in childhood and that the full clinical, hematologic and serological features of the disease occur only in young adults. In view of this information we have undertaken a study to evaluate the serum antibody levels to EBV in various population groups, and in paired sera of cases of selected diseases, especially in young children where a primary EBV infection may be unnoticed. EB virus antibody titers are of particular importance in this age group since even cases of typical infectious mononucleosis may not form heterophile antibodies.

The serum antibody titers were measured by the indirect fluorescent antibody test described by the Henles and their associates.[2,6,7]

The distribution of EBV antibodies in an isolated island population is given in Table 1. St. Paul, a small island in the Bering Sea, has a population of approximately 380 Aleuts in which no cases of infectious mononucleosis or other lymphoproliferative diseases have been reported for the last 40 years during which the population was under constant surveillance by public health physicians. All individuals three years or older had EBV antibodies; the geometric mean antibody titers were stationary in all age groups above three years, and individuals 60 to 70 years old had antibody titers as high

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TABLE 1
SERUM ANTIBODY TITERS TO EBV IN AN ALASKAN ISLAND POPULATION

Age (Years)	No. of Individuals	EBV Antibodies	
		% Positive	Geometric Mean Titer
1-3	11	91	47
4-10	57	100	163
11-20	39	100	163
21-40	45	100	155
41 years and older	37	100	155

as 512 or 1024.

The distribution of antibodies in a mixed population (normal children from an orphanage, infants from well baby clinics and randomly selected normal adults) from the Chicago area is summarized in Table 2.

Maternal antibodies were detected in young infants up to six months of age, and a high percentage (87 to 100%) of individuals four years or older had EBV antibodies. The geometric mean titers were highest in the 1 to 3 year group and remained more or less stationary thereafter with a questionable slight increase in individuals 21 years or older.

Results from the Alaska study indicate that infection occurs on St. Paul's Island uniformly before the age of three years, and this may explain the absence of typical clinical infectious mononucleosis in this population. The constant geometric mean titer in various age groups of both studies indicates that restimulation of antibodies, either from within through an activation of a persistent infection or from without through reinfection, must occur because it is unlikely that such high antibody titers could be maintained without any antigenic restimulation.

A search for conditions under which restimulation might occur was therefore undertaken on the assumption that other infectious diseases might activate a persistent EBV infection, as in other persistent herpes virus infections. The results of this study are presented in Table 3.

Forty-five paired sera from a mumps epidemic in the same Alaskan population, shown in Table 1, were examined. The titers of the individuals ranged from

TABLE 2
SERUM ANTIBODY TITERS TO EBV IN A RANDOMLY SELECTED CHICAGO POPULATION

Age (Years)	No. of Individuals	EBV Antibodies	
		% Positive	Geometric Mean Titer
0-0.5	12	42	16
0.6-0.9	30	0	<2
1-3	68	25	187
4-10	37	94	68
11-20	30	87	75
21-40	25	96	136
41 or older	7	100	105

TABLE 3

EBV ANTIBODY TITERS DURING OTHER VIRAL DISEASES

Disease	No. of Cases	Age in Years	EBV Antibodies	
			Titer Range	Titer Rise
Mumps	45	1.7->60	4-1024	None
Pertussis	20	0.15-2.5	<2-256	1 (<2 to >32)
Serum Hepatitis	7	18-70	32-512	None
Infectious Hepatitis	62	3-35	<2->1024	2 (32-128;<8-32)
Chicken Pox	1	2.5	48	1 (48-512)

4 to 1024 and no titer rise was observed in any of the 45 individuals. The same findings were obtained with paired sera from 20 cases of pertussis with the exception that one child diagnosed as having pertussis actually had a primary EBV infection at the same time. Paired sera from 62 cases of infectious hepatitis (IH) and 7 from serum hepatitis (SH) gave generally similar results. One case of infectious hepatitis probably had a primary EBV infection rather than classical infectious hepatitis, and a four-fold antibody rise in one other IH case could not be further investigated because this was a retrospective study and no other sera were available. In addition, we studied one child who was admitted with a tentative diagnosis of infectious mononucleosis but who had no antibody titer rise to EBV and no heterophile antibodies. This child then developed chicken pox during the course of which the EBV titer rose from 48 to 512, but no heterophile antibodies developed.

From these preliminary data it appears that anamnestic responses of antibodies to EBV can occasionally occur during other virus infections, but more data are needed for a clear understanding of the relationships of EBV antibody restimulation and other febrile illnesses. And what disease, other than typical infectious mononucleosis, is caused by EBV?

We also studied 30 individuals of various age groups admitted to the hospital or emergency room with febrile illnesses. They either showed a rise in EBV antibodies during the course of their disease, or had an already high EBV-antibody titer on admission, of 512 or greater, with subsequently falling titers (Table 4).

TABLE 4

EXPRESSION OF EBV INFECTIONS IN VARIOUS AGE GROUPS

Age (Years)	Total EBV-Positive Studied	Positive For						
		Heterophile Antibodies	Tonsillitis	Lymph-adenopathy	Spleno-megaly	Hepato-megaly	Rash	Lymphocytosis with Atypical Cell
1-5	7	0	6	7	4	3	3	6
6-10	10	2	7	9	3	1	1	6
11-20	10	4	10	10	6	2	0	6
21-30	3	3	2	3	1	1	0	3

None of seven young infants between the age of 1 to 5 years, whose clinical illness was not compatible with infectious mononucleosis, developed heterophile antibodies. Six of these seven had, as the major clinical finding, an abacterial exudative tonsillitis. All had some lymphadenopathy and splenomegaly, and hepatomegaly was observed in four and three respectively. In addition, three infants had a rash, and six of the seven had some hematological abnormalities. The same symptoms were observed in the older individuals, with an increasing number also developing heterophile antibodies and a clinically more typical infectious mononucleosis syndrome.

We are now observing children in an orphanage, noting all minor illnesses and monitoring them for EBV and heterophile antibodies (Table 5). Of 29 children from whom sera were obtained 16 months ago and who were negative for EBV antibodies at that time, 16 children were still in residence a year later when this study began. Six of the 16 had developed EBV antibodies, and only minor febrile illnesses had occurred during the intervening time. One of these showed rising titers and had had an abacterial otitis media just prior to the beginning of serial bleedings for this study. Three months later, eight of the ten negative children were still available for study, and five of these had developed EBV antibodies, but none had developed heterophile antibodies. Again only minor episodes of one-to-three day fevers and mild upper respiratory infections had occurred in the interval.

Last, but not least, three cases of American "Burkitt's" lymphoma from our own hospital had EBV titers of <2, and the third case had a stationary titer of 1:8.

SUMMARY

First, we have confirmed the association of EBV with infectious mononucleosis, which we would prefer to call self-limited lymphoproliferative disease, and have further shown that, particularly in young children, cases of typical disease may not form heterophile antibodies. Second, we found that in a controlled population where EBV infection occurs in all individuals before the age of three years, typical infectious mononucleosis does not occur. However, high antibody titers can be found throughout life, indicating that antigenic restimulation must occur not infrequently. Preliminary data indicate that cases diagnosed as exudative tonsillitis, pertussis, otitis media, infectious hepatitis or other minor febrile illnesses in childhood, generally with lymphadenopathy with or without hematological changes, actually represent primary EBV infections. Antigenic restimulation of antibodies, probably through an activation of a persistent EBV infection, can occur during other viral infections. Third, in three cases of American lymphoma, which clinically and histologically were diagnosed as Burkitt's lymphoma, no association with EBV could be made.

TABLE 5
EBV ANTIBODY TITERS IN
A CHICAGO ORPHANAGE

Time of Bleeding (Years)	Age* (Years)	No. of Individuals	EBV Titer	
			<2	>2
0	0.5-1	29	29	0
1	1.5-2	16	10	6
1.25	1.8-2.3	8	3	5

*Age at time of bleeding

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A COMPARISON OF SERUM GROWTH HORMONE RESPONSES TO VARIOUS STIMULI FOR EVALUATION OF PITUITARY FUNCTION

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INTRODUCTION

The advent of the radio-immunoassay for human growth hormone (HGH) levels in serum has made available a sensitive and useful tool for the laboratory assessment of pituitary function. While the elevated serum levels of HGH which occur in acromegaly or gigantism are easily detected, the normal fasting levels are frequently so low as to be indistinguishable from those seen in hypopituitarism. Consequently some type of stimulus must be applied which causes an elevation of HGH levels in the normal subject, but to which the subject with hypopituitarism does not respond similarly. The rise in serum HGH levels in response to insulin-induced hypoglycemia, as demonstrated by Roth *et al.*,[1] has been successfully used for the evaluation of hypopituitarism, but the hypoglycemia produced during the test is somewhat uncomfortable and potentially hazardous. An intravenous infusion of arginine, although also successful in elevating serum levels of HGH in normal subjects, is difficult to administer to children and in an outpatient setting. Therefore, it was felt, a need existed for an oral method of stimulating HGH secretion which would both simplify and lower morbidity of the testing procedure, while preserving the meaningful levels of elevation obtained otherwise.

This study was undertaken to reproduce the effects of insulin-induced hypoglycemia and intravenous arginine in two normal subjects. In addition oral methods for producing elevations in serum HGH were evaluated, utilizing broths containing protein hydrolysates and an arginine solution.

MATERIALS AND METHODS

All tests were performed in duplicate on two 23-year-old male medical students, each weighing 65 kg. They were in excellent health, with normal thyroid function and glucose tolerance, and there

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was no history of systemic or endocrinologic disease. Oral 1-arginine was administered as the monohydrochloride salt (Pfannstiel) in a dose of 0.5 gm/kg, on one occasion in water and on another in a sugar-free cola, ingested over a period of about three minutes. Intravenous arginine (Cutter) was administered in the same dose as a 10% solution of the monohydrochloride salt infused over a thirty-minute period. Crystalline insulin (0.1 units/kg) was administered rapidly by intravenous route. Bovril®, a beef boullion composed of beef and vegetable protein hydrolysate, was given orally in a dose of 30 cc. in 175 cc. of water. Monarch® Boullion Cubes, a vegetable protein hydrolysate, were given as two cubes dissolved in 240 cc. of water. Blood specimens were drawn at thirty-minute intervals, and the sera frozen until time of analysis. Blood sugar determinations were done on specimens drawn simultaneously with HGH samples during insulin-induced hypoglycemia.

Blood sugar results were determined on the Technicon Autoanalyzer by a modification of the method of Hoffmann.[2] HGH levels were determined by a modification of the double antibody method of Morgan.[3]

RESULTS

The results of different stimulation tests are presented in combined graphs and tables. Fig. 1 shows that intravenous arginine produced a significant elevation of HGH levels which was evident at the end of the thirty-minute infusion. There was a further increase of the mean value over the next thirty-minute interval. This was followed by a rapid decline and then a gradual tapering off, so that by the end of the two-and-one-half hour test the mean HGH level had returned to baseline levels. The accompanying table shows that subject AAH (one of the authors) had a two-and-one-half hour value of 10.7 mμg./ml. This is a brisk response for an adult male, but the arginine appears to have had a sustained effect through this test, as evidenced by the slow decline of HGH levels. Oral arginine failed to show a consistent significant elevation of the mean level, and also produced an almost immediate diarrhea.

Fig. 2 shows the results obtained with insulin-induced hypoglycemia. The zenith of HGH levels was apparently reached one hour post-injection. Significant decreases of greater than half of fasting blood sugar values, accompanied by symptoms of hypoglycemia, were obtained in all instances. Blood sugars reached their nadir by thirty minutes in all but one of the tests. By the end of the testing period, homeostatic mechanisms had restored blood sugar to normal levels, but the HGH remained significantly elevated. Glucose was administered at the termination of each test.

Table I shows the results obtained with various oral boullion stimuli. Tests with Bovril were initially run over a two-day period, and a significant elevation was found in the terminal specimen of the second day. Unsure whether this was laboratory error or the delayed effect of high-protein ingestion, we repeated the procedure. One subject (HND) failed to respond to the repeated stimulus, but the other (AAH) showed an even greater elevation in the terminal specimen on the second day. This value was attributed to exercise prior to drawing the sample and must be considered invalid. Monarch Boullion Cubes failed to give any significant response.

DISCUSSION

Failure of insulin-induced hypoglycemia to initiate an increase in serum levels of HGH has been described by Frantz and Rabkin[4] as the most sensitive indicator of pituitary hypofunction. As has been stated, existing intravenous methods of elevating serum HGH levels are inconvenient in an outpatient setting or associated with an inherent morbidity, and it would be desirable to obtain an oral agent capable of producing comparable elevations.

Previously, regardless of the stimulus, the highest responses in normal subjects have been detected in children, adult females, and estrogen-pretreated adult males.[5] The question arose as to whether the subjects in this study would react as untreated adult males and whether responses would be brisk enough to warrant labeling them positive. A notable rise in serum HGH levels was obtained with intravenous infusions of arginine or insulin.

INTRAVENOUS ★- - - -★

Time	0	30	60	90	120	150
$_1$AAH	1.4	18.2	25.3	23.1	25.5	10.7
$_2$AAH	10.7	23.3	27.5	11.3	5.3	2.9
$_1$HND	1.4	20.8	18.7	5.3	2.0	1.6
$_2$HND	1.5	26.8	41.2	8.1	3.8	2.3
MEAN	3.8	22.3	28.2	12.0	9.2	4.4
S.D.	4.3	3.7	9.5	7.8	11	4.2

ORAL ●——●

Time	0	30	60	90	120
AAH	1.8	8.4	3.9	1.8	2.5
AAH	5.4	2.4	<1.25	<1.25	<1.25
HND	1.5	1.5	1.6	1.6	1.3
HND	1.25	4.25	1.4	1.4	<1.25
MEAN	2.5	3.4	2.0	1.5	1.6
S.D.	1.9	3.4	1.3	0.2	0.6

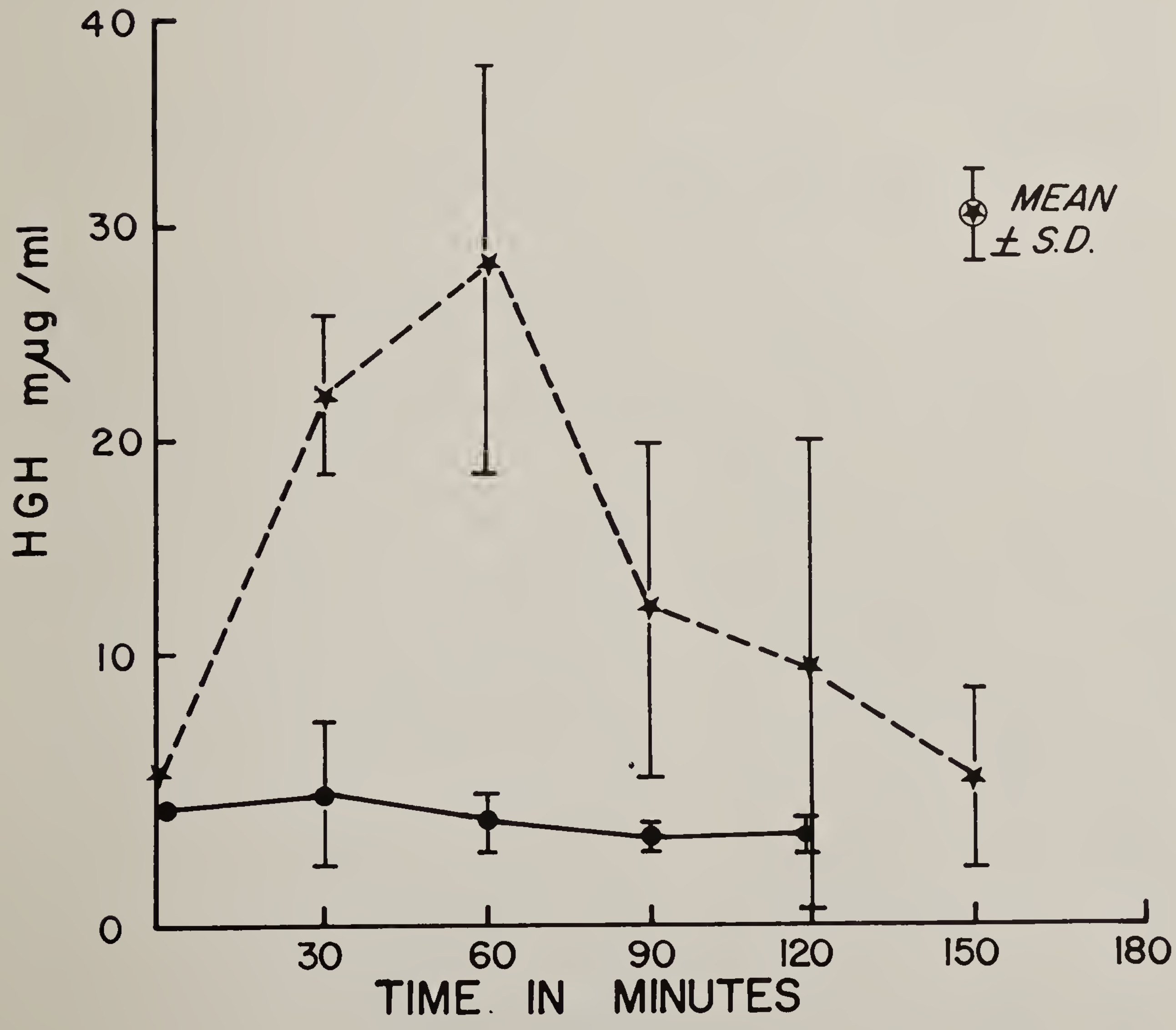

Fig. 1. Serum H G H responses to intravenous and oral arginine.

	TIME	0	30	60	90	120
AAH 1	HGH	1.6	4.7	>80	64	33
	BS	80	30	46	66	83
AAH 2	HGH	1.55	4.0	65	60	35
	BS	80	27	50	62	81
HND 1	HGH	2.5	2.7	84	83	62
	BS	71	27	32	50	59
HND 2	HGH	2.4	11.5	92	92	42
	BS	69	35	30	47	62

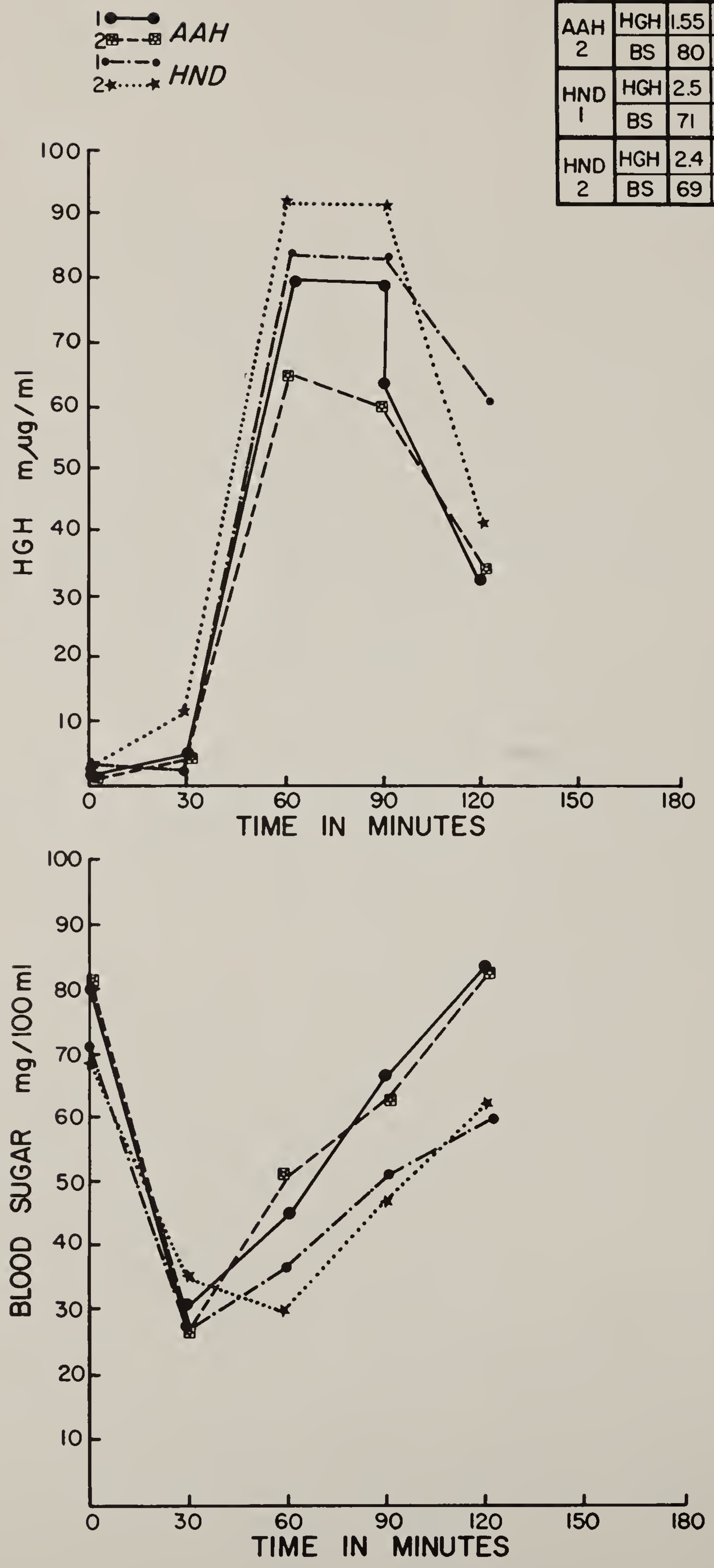

Fig. 2. Serum H G H responses to insulin-induced hypoglycemia.

TABLE I

HGH (μmg/ml) RESPONSE TO ORAL BOULLION—MONARCH® and BOVRIL®*

	Time (min.)	0	30	60	90	120
MONARCH®	AAH	7.8	3.2	1.3	1.3	1.9
	AAH	2.3	1.4	2.8	1.9	1.6
	HND	<1.25	<1.25	<1.25	1.55	7.8
	HND	<1.25	<1.25	<1.25	<1.25	<1.25
	MEAN	3.2	1.8	1.7	1.5	3.1
BOVRIL® (1st two days)	AAH	1.6	1.6	1.5	1.6	1.7
	AAH	2.2	1.6	1.7	2.2	19.0
	HND	1.9	1.6	1.6	1.7	1.8
	HND	1.7	1.4	1.5	1.8	16.1
	MEAN	1.9	1.6	1.6	1.8	9.7
BOVRIL® (2nd two days)	AAH	<1.25	1.5	<1.25	<1.25	3.3
	AAH	<1.25	<1.25	<1.25	2.1	40.0
	HND	<1.25	<1.25	<1.25	<1.25	<1.25
	HND	<1.25	<1.25	<1.25	<1.25	<1.25
	MEAN	<1.25	1.31	<1.25	1.46	11.5

*Registered Trademarks

These results clearly confirm that both of these agents are effective stimuli for growth hormone secretion, while they also imply that 23-year-old males may still respond in a lively manner.

Insulin-induced hypoglycemia produced the highest levels of serum HGH in this study, with values of greater than 80 mμg./ml. in both subjects, on several occasions. The stimulus for these elevations is probably mediated through depletion of central nervous system intracellular levels of glucose. This has been substantiated by administration of 2-deoxyglucose, a nonmetabolizable substance which is taken up by the cell but effectively blocks the breakdown of glucose by the glycolytic pathway, and even in the presence of high levels of plasma glucose causes an elevation in serum HGH levels.[6]

The intravenous arginine infusion produced somewhat lower, although respectable elevations in growth hormone. Such elevations have been shown to be independent of arginine's effect on secretion of insulin, and arginine causes slight hyperglycemia rather than hypoglycemia.[7]

Only slight elevations in serum HGH occur after ingestion of high-protein meals.[8] The reason for these elevations is not known, but one could speculate that amino acids and small peptides absorbed from the gut and subsequently present in the plasma may be implicated as the intermediate stimulatory agents. Recently, Bovril was employed as a stimulus. Elevations in serum HGH of 20 to 25 mμg./ml. were noted at sixty minutes in children and adult females.[9] It was undertaken in this study to duplicate these previously noted elevations, first with Monarch Boullion Cubes and then with Bovril, but no reproducible elevations were obtained.

Further attempt at oral methods of stimulation with arginine solutions resulted in severe diarrhea within a short time following ingestion, leading to lack of absorption of the amino acid and insufficient concentration in the blood to initiate a response.

The oral tests do not appear to provide effective stimuli to growth hormone secretion, at least in two young adult males. Contrary to a recent editorial in *The Lancet*[10] regarding Bovril, these tests probably should be considered relatively ineffective procedures, particularly in adults.

Intravenous arginine seems to provide the optimum balance between low patient morbidity and significant results. However, occasionally a single test will not produce an accurate evaluation for the investigator. This consideration is pertinent when no elevation of HGH levels is observed with intravenous arginine. In such a case, insulin-induced hypoglycemia might be considered as a secondary measure for supportive evidence regarding pituitary hypofunction.

SUMMARY

Comparisons of serum growth hormone responses to various oral and intravenous stimuli were made in two healthy young male subjects. The oral agents tested were ineffective as stimulators of growth hormone secretion. Hypoglycemia due to intravenous insulin injection was the most potent stimulus. However, intravenous arginine was effective, if not as potent a stimulus, and was the best tolerated.

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REVIEW ARTICLE

COMPUTERS IN PSYCHIATRY

James F. Scharf

The purpose of this paper is to present briefly some of the existing applications of computer technology to the field of psychiatry. The following areas will be considered: (1) medical literature storage and retrieval system, (2) analysis of data from medical records, (3) automated psychological testing, and (4) automated psychiatric histories and clinical observations.

NATURE OF COMPUTERS

A computer is an automatic device for the rapid processing of data. There are two types of computers, analogue and digital. The type used mainly in medicine is the digital computer, which operates in terms of discrete numbers or letters, commonly called digits. In the electronic digital computer, alpha-numerical digits are coded as small groups of binary digits, each represented as a circuit in an *on* or *off* state.

A computer system is an interrelated group of components acting as a unit. These components can usually be categorized as *Input/Output*, *Secondary Memory-Input/Ouptut*, and the *Central Processing Unit* (CPU). The CPU can be considered to consist of additional interrelated functioning systems: memory, arithmetic, logic, and control. The entire system is under the direction of the control section of the system. Input devices such as punched cards or magnetic tapes feed pertinent data into the system. The CPU processes this data in a predetermined fashion or according to its program, which is a set of instructions stored in its memory. The results come out as processed data, printed out in numbers or words by the output device. Thus, a computer system can communicate, store and recall, translate, rearrange, summarize, and compute. However, no matter how complicated or sophisticated a computer system is, it is still a tool.

Like all good tools, the computer is an extension of some of man's own abilities. Specifically: (1) computers are able to read and write; (2) computers are able to memorize information including their own programs in coded form for long or short periods of time; (3) computers are able to follow instructions, performing arithmetical and logical operations; and (4) computers are able to operate rapidly and accurately.[1] Advantageous also are activities in which a computer need not engage: "It has no sex life, no prejudices, no political convictions, no taste for music or the graphic arts, no anxieties, no wife, no income tax, and does not have to make water or go to stool."[2]

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There are also disadvantages. Being a machine, a computer will do only what it is instructed to do. It lacks inspiration, capacity to improvise, and any form of creative ability. It is also subject to computer error and *down time* (periods when the computer will not work).

EXAMPLES OF CURRENT COMPUTER USE

Medical Literature Analysis and Retrieval System (MEDLARS): In January of 1964, the National Library of Medicine put into operation a computerized bibliography system called MEDLARS. The National Library of Medicine receives more than 16,000 journals each month, of which 3,600 are considered to be of significant consequence to the medical community. From these 3,600 journals, about 165,000 articles per year have been indexed for inclusion in MEDLARS. It is projected that by 1970, MEDLARS will have to index some 250,000 articles per year.

The preparation of this paper is an example of how MEDLARS may be used by an individual. With the assistance of Mr. William Kona, of the Rush Medical Library of Presbyterian-St. Luke's Hospital, I sent a request to the National Library of Medicine for a MEDLARS search on the topic, *Computer Use in Psychiatry and Psychology.* That request resulted in NLM-BSD Search Number 005429, which produced a bibliography containing 80 citations. Each citation was printed on a 3x5 card and contained the title, author, and journal reference for a pertinent article. In addition to providing a bibliography upon request, the National Library of Medicine conducts periodic literature searches on a variety of subjects, using MEDLARS.

Professional Activity Study (PAS). Information from medical records also is being utilized in electronic data processing. The largest and most comprehensive computerized medical record system is conducted by the Commission on Professional and Hospital Activities (CPHA) in Ann Arbor, Michigan. Approximately 850 hospitals in the United States take part in the Commission's Professional Activity Study which handles almost nine million case abstracts per year. Although the chief current use of PAS reports is in connection with bed utilization review, this huge data file also lends itself to research of clinical interest.[3]

Working from the patient's regular clinical record, the hospital's medical librarian answers on a separate form, either with pencilled checks or digits, the nearly 200 questions on the PAS Case Abstract. This information is subsequently coded and put onto magnetic tapes for computer analysis. A participating hospital receives six reports each month and an additional ten reports every six months for its own use. The case abstract data, stored on magnetic tapes in the CPHA data bank, is used by in-house researchers and outside institutional, private, and governmental medical investigators under strict rules which preserve the anonymity of hospitals, physicians, and patients.

In 1967, V. N. Slee noted: "Actually, the surprising thing about the electronic data system revolution in hospital practice is that hospital data are being used so little in so many areas of clinical and academic medicine . . . The computerized data systems came upon medicine so suddenly that the medical schools never had time to structure and offer courses in the philosophy, perspectives, and techniques of information management in their basic curricula."[4]

Minnesota Multiphasic Personality Inventory (MMPI). One of the first physicians to use computers in medicine was Dr. Howard P. Rome, of the Mayo Clinic, who helped to develop a computerized program of scoring and reporting the Minnesota Multiphasic Personality Inventory which had been given routinely to medical and surgical patients at the clinic. To date, more than 250,000 MMPI records have been put on computer tapes at the Mayo Clinic.[5]

The MMPI, developed by Hathaway and McKinley at the University of Minnesota, consists of 550 *yes-no* questions and is the most commonly used structured personality test in the world. Its format deduces information about the patient from his personally filed responses to the 550 standard statements. These responses constitute a typological profile based on a binary system of *yes-no* judgments which make, not only the mechanization of its administration, but also the tabulation of its scores, a readily programmed machine procedure. After a patient has taken the test, the answer sheet is scored and interpreted by the computer system which prints out a report containing an analysis of the 12 MMPI scales and standard interpretative statements concerning the scales and profile, corresponding to interpretations usually used by psychologists. The total time required for computer processing is 35 seconds.

Mental Status Schedule and Psychiatric Status Schedule Subject Form (MSS-PSS-S). Other groups of investigators have been working on computerizing data derived from psychiatric interviews and clinical observations. One such group is Dr. Robert L. Spitzer and his associates in the Department of Psychiatry of Columbia University. As they have so aptly stated: "The mental health professions are aware of the need for objective, reliable and valid measures of current and past psychopathology. Over the last 20 years, numerous instruments and recording forms have been developed with varying degrees of usefulness for psychiatric research. In an attempt to overcome some of the limitations in existing procedures, we have developed an integrated group of instruments, forms, techniques, and computer programs to assess aspects of the psychiatric status, history, and social background of subjects in a quantitative manner. The instruments are primarily designed to improve the research value of clinical judgments of psychopathology based upon a psychiatric interview of a subject or other informant. Although all the procedures . . . can be used for the clinical evaluation or description of the individual subject, their major use is in the research comparison of groups of patients or nonpatients."[6]

Among such instruments are the MSS and the PSS-S. The MSS is a ten-page booklet in step-down format which contains an interview schedule and a matching inventory of 248 dichotomous (*present-absent*) items that focus upon signs and symptoms associated with neurotic, psychotic, and organic disorders. The booklet is used repeatedly, with a separate score sheet for each patient. Each score sheet has a carbon copy and serves simultaneously as a permanent clinical record and as a form for automated data processing. All data are obtained and recorded during the examination of the patient, so that the evaluation is completed at the same time as the interview. The entire evaluation generally takes from 20 to 50 minutes.

The data are then analyzed by a computer according to a factor-analytically-based scoring system which yields standardized scores on 3 macro and 13 factor scales that correlate well with other measures of similar dimensions derived from various rating and self-report procedures. An expanded version of the MSS, is the PSS-S which, in addition to dealing with neurotic, psychotic, and organic disorders, covers signs and symptoms associated with alcoholism, drug addiction, and sociopathic behavior, as well as role-functioning.

The PSS-S has a format similar to that of the MSS, but has an inventory of 492 dichotomous and checklist items. The 248 items of the MSS are interspersed within the PSS-S, permitting the extraction of MSS scale scores from data collected using the PSS-S. The flow of the interview is similar to that in the MSS except for the inclusion of the additional areas. The entire evaluation takes from 35 to 75 minutes, depending upon the amount of psychopathology, verbal productivity, and cooperation. The PSS-S has 17 factor-based scales, 6 role-impairment scales, and 18 supplemental scales. However, differing from the MSS, some of the items are scored with unit weights, permitting clinical judgments of severity. The three sets of scales have been used in a computer program, DIAGNO-II, which yields a psychiatric diagnosis for each subject.

The validity of this use of the scales has been tested against clinical judgment, with about 50% agreement between computer and physician. This is approximately the same agreement as between physician and physician. Information concerning the percentage of agreement of DIAGNO-II was related by Dr. Spitzer at the May, 1968 meeting of the American Psychiatric Association, in Boston. He explained that they would have to modify their computer program to conform to the recently revised nomenclature. However, even though they modify their computer program, I believe that this way of making a psychiatric diagnosis will be more difficult in the future because the new Diagnostic and Statistical Manual of Mental Disorders (DSM-II) encourages the use of multiple psychiatric diagnoses.[7] Albeit, this possible "difficulty" does not lessen the increasing usefulness of computers in psychiatry.

Institute of Living. Another group using computers clinically is the one headed by Dr. Bernard C. Glueck, Jr., at the Institute of Living, a private psychiatric hospital in Hartford, Connecticut. This group recognizes the value of utilizing nurses' notes. In many institutions, the observations of nursing staff are not charted, or are charted in a sketchy manner. One significant reason for the lack of good nursing notes is failure on the part of physicians to read them—because they are not informative, the handwriting is not legible, they are too lengthy, or for other reasons. To render such observations meaningful, known, and useful to the therapist, Dr. Glueck and his group have developed computerized observation reporting systems for the nursing staff and the occupational therapy staff. These systems make possible the collection, storing, distribution, and analyzing of observations from both departments.

The basic method for collecting and reproducing these observations consists of sets of descriptors of behavior from which personnel select those applicable to the patient they are describing. Entries are made on special forms which are then processed by an optical scanner and a computer, producing automated notes in narrative form. In addition, they utilize a form called a Special Behavior Report which is processed in the same manner.

Their work has shown that automated nursing notes perform the following functions: (1) make the recording and transmitting of information more efficient, (2) improve staff observations of patients, (3) retain all of the currently used, clinical information present in the traditional form of charting, (4) provide standard terminology meaningful to personnel, (5) provide standard terminology to minimize interhospital differences, (6) provide data for statistical analysis of behavior, (7) furnish useful information in an acceptable format and size, and (8) assist in the education of hospital personnel at all levels.[8]

With respect to interhospital comparisons, arrangements were made to use the above-mentioned forms in certain wards at the nearby Middletown State Hospital and in the psychiatric wards of Hartford Hospital. Also of importance is that the employment of these forms significantly changes the relationship of research to regular hospital routines, because computer research is able to analyze data accumulated in day-to-day work.[9] In addition to these forms, the Institute of

Living uses computerized psychological testing, namely the Minnesota-Hartford Personality Assay (M-HPA) and the MMPI. Their computerized MMPI program was developed with the assistance of Dr. Howard P. Rome, of the Mayo Clinic.

In discussing the computer, Dr. Glueck has stated: "We look upon the computer simply as another laboratory instrument. It enables us to specify and quantify behavior—in effect, to measure behavior. And since it is behavioral modification that is our main therapeutic job, we have for the first time a useful laboratory technique."[9]

COMPUTERIZED PSYCHIATRIC HISTORIES

At the Rockland State Hospital, Orangeburg, New York, Dr. Nathan S. Kline and his associates have developed a computer program known as Systematic, Complete, Retrievable, Interlingual, Brief, Expandable (SCRIBE) for use with a standardized questionnaire, the General Purpose Psychiatric Questionnaire (GPPQ). After the 49 items in the brief multiple-choice GPPQ have been checked, the necessary research information is collected and stored for future use, while a computer using the SCRIBE program produces a narrative history corresponding to the questionnaire in a matter of a few seconds. The GPPQ was designed to facilitate comparisons (1) among patients in the same hospital, (2) among patients in different hospitals, (3) between patients in hospitals and patients in private practices, and (4) between patients in a variety of private practices.

An interesting feature of this program is that it was also designed to be used in many countries throughout the world. The output of SCRIBE is multilingual, even though no language translation by computer is involved. Since the computer reads only punched card codes rather than words, and answers to the GPPQ are standardized, the computer can produce a narrative printout in any language for which it has been programmed. The GPPQ form is available in eight languages, and computer output is available in four languages: English, German, Spanish, and French.[10]

Dr. Kline and his group also have designed similar forms for both the mental status examination and the developmental history. These two forms, combined with the GPPQ, are fed into a computer which has a different program, known as Narrative Output Vocabulary Editing Language (NOVEL), by which the computer is able to produce a routine psychiatric case history from the data on all three forms. As with SCRIBE, computer printout from NOVEL is available in several languages.

The new, second edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-II), published in 1968, by the American Psychiatric Association indicates, in the chapter on statistical tabulations, the forthcoming influence of the use of computers in psychiatry: "The next few years will undoubtedly witness further progress in this field as a result of the increasing use of automated data-processing methods in mental hospitals, general hospitals, and other facilities where psychiatric services are provided. These methods will make it possible to introduce further improvements into the management and use of records for improved patient care and facilitate greatly the preparation of more extensive statistics on the diagnostic and related characteristics of the patients under care in psychiatric facilities."[7]

SUMMARY

This paper has briefly presented some of the existing applications of computer technology to the field of psychiatry. It is expected that these applications will increase our knowledge and understanding of emotional disorders and improve the quality of psychiatric care.

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10. Laska, E., *et al.*: SCRIBE-A Method for Producing Automated Narrative Psychiatric Case Histories. Amer. J. Psychiat., 124: 82-84, 1967.

ABSTRACTS

OF PUBLICATIONS BY THE STAFF

Lepper, Mark H., Shioura, Norio, Carnow, Bertram, Andelman, Samuel, and Lehrer, Leah: Respiratory Disease in an Urban Environment. *Indust. Med. Surg. 38:36-41, April, 1969.*

Stratification of the Chicago population by socioeconomic criteria and exposure to varying concentrations of SO_2 have indicated that each of these environmental variables may play a role in deaths from a variety of respiratory diseases considered together. This finding is similar to that reported by Zeidberg in Nashville. Since the magnitude of differences attributed to these environmental factors is small compared to those for age, sex, and race of the host, it is not clear that the latter factors are sufficiently controlled. Analysis of the data for adults only, and only the white population, weakened rather than strengthened the evidence of association.

Carnow, Bertram W., Lepper, Mark H., Shekelle, Richard B., and Stamler, Jeremiah: Chicago Air Pollution Study: SO_2 Levels and Acute Illness in Patients with Chronic Bronchopulmonary Disease. *Arch. Environ. Health, 18:768-776, May, 1969.*

A study was done to determine whether a dose-effect relationship exists between levels of pollution as measured by SO_2 and acute morbidity from respiratory disease in patients with chronic bronchopulmonary disease. For this purpose a Registry of 561 patients with chronic bronchopulmonary disease was established. A graded pulmonary disease classification was developed, based on severity of symptoms; a daily personal pollution index was established for each patient, based on SO_2 levels in his square mile of employment and residence; and a daily calendar was maintained to record acute respiratory illness. In patients aged 55 and over with grade 3 and 4 bronchitis, an increase in the acute morbidity rate was found in association with increased levels of SO_2. This increase in illness rate was recorded for the day of exposure and more consistently for the day following exposure.

Carton, Robert W. and Wong, Ruth: Multifocal Fibrosclerosis Manifested by Vena Caval Obstructions and Associated with Vasculitis. *Ann. Intern. Med., 70:81-86, Jan. 1969.*

Multifocal fibrosclerosis is the generic term currently used to describe a group of fibrosing conditions including chronic mediastinal fibrosis and retroperitoneal fibrosis. A 29-year-old brewery worker with obstructions of his superior and inferior vena cava due to mediastinal and retroperitoneal fibrosis was followed for 3 years until his death from hemoptysis. He also suffered from peripheral ulcerations due to disseminated vasculitis. Vasculitis has not been observed previously associated with chronic fibrous mediastinitis, although it has been found together with other members of the mutifocal fibrosclerosis group (retroperitoneal fibrosis and sclerosing cholangitis).

It is hypothesized that inflammation of the blood vessels may lead to multifocal fibrosclerosis or may be present in association with this condition in response to the same primary stimulus.

Steinwald, Osmar P. Jr., Doolas, Alexander, Southwick, Harry W.: Pheochromocytoma. *Surg. Clin. N. Amer., 49:87-98, Feb. 1969.*

Pheochromocytoma and an approach to its treatment have been discussed. Quantitative measurement of metabolites of catecholamines in the urine has been shown to be the safest and most reliable test for screening large numbers of patients. Screening tests should be more widely employed, because some patients with pheochromocytoma may present with minimal signs and a paucity of symptoms. Finally, success of the surgical extirpation of pheochromocytoma depends directly on adequate pretreatment with alpha blocking agents and careful intraoperative and postoperative care. During the operative procedure, care includes the use of halogenated hydrocarbons as the anesthetics of choice, judicious use of both alpha and beta blocking agents, and adequate maintenance of blood volume.

Ecanow, Bernard, Balagot, Reuben C., and Bandelin, Valerie: Effect of Suspended Particles on the Gas-Absorbing Ability of Lung Surfactants. *Amer. Rev. Resp. Dis., 99: 106-108, Jan. 1969.*

The effect of powder particles on the ability of lung surfactant to absorb a nonpolar gas, halothane, was studied by placing powdered sulfamerazine upon concentrated and dilute solutions of lung surfactant and upon solutions containing no surfactant. Samples of these solutions and of solutions containing powder were placed into a tonometer. The space above the samples contained halothane gas. The rate of gas disappearance from the space was followed by withdrawing aliquot gas samples and measuring halothane concentration by means of gas chromatography.

It was found that concentrated surfactant solution without powder absorbed the gas rapidly and to the greatest extent. Concentrated surfactant solution containing suspended powder absorbed gas to a lesser extent than solutions without powder. The rate and extent of gas absorption in both cases indicate the presence of micelles in the surface. A decrease in surfactant concentration lessened the amount of gas absorbed, and it was concluded that fewer micelles were present. The addition of powder to the dilute surfactant solution appeared to result in the concentration of surfactant upon the interface. Evidence for this was the increased absorbing ability of the suspension as compared to the solution.

Samples with an aqueous surface containing only trace amounts of surfactant absorbed little or no gas.

Balagot, R. C. and Bandelin, V. R.: Comparative Evaluation of Some DC Cardiac Defibrillators. *Amer. Ht. J., 77:489, April 1969.*

Conversion of ventricular fibrillation and certain other types of cardiac arrhythmias with an electronic defibrillator has become an accepted therapeutic procedure. The superiority of direct current (DC) over alternating current (AC) defibrillation has become established. Because of this, there has been a proliferation of available DC defibrillators.

This study compared the electrical characteristics of four defibrillators with respect to the following variables: (1) the energy delivered across the electrodes as a function of the selected energy setting in watt-seconds; (2) the variation of energy delivered as a function of the load or body resistance; (3) a comparison of energy stored in the capacitors against energy delivered; (4) a comparison of energy setting versus peak current for different body loads; and (5) a comparison of energy selected against peak voltage for various body loads.

Three of the defibrillators evaluated were found to deliver substantially less energy (20% to 78% less) than indicated by the energy selector controls, when discharged to a range of resistance usually encountered clinically.

The energy delivered by all LC circuit defibrillators varies as a function of body resistance. Unless calibrated in *delivered energy* rather than *stored energy*, the output may vary widely among defibrillators of different manufacturers.

All units tested have a characteristic internal loss of energy, which is superimposed on the loss due to mismatch with body resistance.

The single LC defibrillators, with waveforms of three or four milliseconds in duration, achieve extreme peak voltage and current amplitudes at high energy level selection. These amplitudes have previously been shown to be deleterious.

Since resistance measurements are generally not available to the clinician, and conversion tables relating stored energy to delivered energy would be awkward at best, some standardization of the outputs of defibrillators is indicated. It is suggested that calibration of the energy setting to indicate *delivered energy* to a 75 ohm load be adopted.

Elstein, Arthur S. and Van Pelt, John D.: Structure of Staff Perceptions of Psychiatric Patients. *J. Consult. Clin. Psychol., 32:550-559, 1968.*

Employing the "implicit personality theory" method suggested by Cronbach for studies of person perception, a 63-item rating scale, covering cognitive functioning, emotional tone, interpersonal relations, and prognosis, was used in 2 concurrent studies of the categories employed by psychiatric staff in the perception of patients. In study 1, 12 patients were assessed by 9 judges; in Study 2, 29 patients were assessed by 6 judges. Separate factor analyses of the 2 sets of ratings resulted in identifying 5 factors common to both studies as well as factors unique to each. The common factors were interpreted as: Need for Staff Control, Prognosis, Dependency vs. Counterdependency, Dysphoric Psychopathology, and Favorability.

Van Pelt, J. D., and Elstein, A. S.: Assumed Similarity in Staff Perceptions of Psychiatric Patients. *J. Clin. Psychol., 25:96-97, Jan. 1969.*

Psychiatric inpatients were described by members of psychiatric unit staff using a *Q* sort. In addition, each rater was asked to predict the way he thought some other member or group of the staff would describe the patient (the similarity method). The median real similarity correlation was .33 and the median predictive accuracy correlation was .30, while the median correlation of assumed similarity was .63, approaching the reliability of the instrument. The domination of prediction so greatly by this response tendency is viewed as touching on the rater's need to see his relationship with the patient supported by the belief that the rest of the treatment team shares his point of view.

Dicken, Charles and Van Pelt, John: Further Evidence Concerning Acquiescence and the MMPI. *Psychol. Reports, 20:935-941, 1967.*

Ninety-four normal and one hundred schizophrenic subjects were tested with the MMPI and with a version of the MMPI composed of items reversed in meaning. Comparison of original and reversed items and scales indicated little if any of the variance of the original can be attributed to acquiescence response style. Several item-reversal studies of the MMPI are reviewed and a high degree of consistency is noted.

Van Pelt, J. D., Bock, R. D., and Dicken, C. F.: Methodological Implications of Content-Acquiescence Correlation in the MMPI. *Psychol. Bull., 71:127-139, Feb. 1969.*

In this paper we attempt to resolve differences remaining between opposed points of view on the importance of acquiescence response bias in the MMPI. On the basis of results in the literature and new data presented herein, we conclude that some variance atrributable to acquiescence is undoubtedly present in the MMPI scores, but that it is small relative to the content variance. We find, however, independently corroborated evidence that the acquiescence tendency is substantially correlated with first factor content in the MMPI. Our calculations show that this correlation can account for many of the properties of the MMPI scales which other workers have attributed directly to acquiescence. In particular, we find that, in the presence of acquiescence-content correlation, a relatively small component of variation due to acquiescence has an appreciable effect on the (1) psychometric characteristics of the scales, (2) the selection of items for special scales, and (3) the correlation of certain MMPI scales with independent measures of acquiescence.

Laufs, Rainer, Holmes, Albert W., and Deinhardt, Friedrich: Method of Human and Monkey Liver Organ Culture and its Successful Use for Propagating DNA and RNA Viruses. *Clin. Res., 16:460, Oct. 1968.*

Cultivation of adult marmoset and human liver in pieces approximately the size of single lobules is described. Cells lived for 3 weeks as shown by their histology. Their metabolism was autoradiographically and biochemically investigated. The cultured liver tissue showed a high RNA and protein synthesis, which changed only slightly between the 1st and 15th day of culture. Several DNA and RNA viruses multiplied in the hepatocytes to high titers and produced characteristic cytopathic effects even if inoculated as late as 10 days after explantation. The ability of cultured liver tissue, as shown in these studies, to maintain its structure and metabolism, and to support virus multiplication has potential in studies of human hepatitis viruses.

Edwards, L. D., Balagtus, R., Lawrence, R., Eisner, L., Lowe, P., Lepper, Mark H.: Antimicrobials, Infections, and the Nurse Epidemiologist at Presbyterian-St. Luke's Hospital, Chicago. *Proc. 4th Joint Mtg., Clin. Soc. & Comm. Off. Assn. U.S.P.H.S., June, 1969,* p. 12 (Abstract).

A twelve day period of intensive surveillance of hospital-associated infections was carried out by three physicians from the infectious disease section and two nurse epidemiologists. Data collected by the physicians was compared in detail to data collected by the nurses, and the reliability of the latter was substantiated.

Other findings indicate approximately 60% of patients on the medical and surgical units receive antimicrobials during their hospital stay (42% of patients on medicine and 73% of patients on surgery). On the same services 35% of patients had hospital-associated infections (28% medical and 41% surgical). The community onset infection rate was 17% and the hospital onset infection rate was 19%.

The hospital-associated infection rate on obstetrics was 8% and on pediatrics (excluding nursery patients) was 25%. The need for follow-up on discharged obstetric patients and newborns for detection of possible hospital-onset infections is discussed. Plans for more intensive surveillance of hospital-associated infections with computerization of data and monthly reports for all physicians is also discussed. Suggestions are given for decreasing our hospital-onset infection rate and for curtailment of antimicrobial use.

Hillis, W. D., Holmes, A. W., and Davidson, V.: Serologic Characterization of Adenoviruses Isolated from Chimpanzees Associated with Viral Hepatitis. *Proc. Soc. Exptl. Biol. Med., 129:366-369, 1968.*

Chimpanzees and certain other nonhuman primates have been implicated in recent years in the transmission of viral hepatitis to their human caretakers and to other contacts. Mounting epidemiologic evidence for such transmission is documented by more than 100 human cases of the disease. This evidence supports the postulate that certain nonhuman primates act as carriers of the etiologic agent or agents of human viral hepatitis or of a disease so closely related as to be indistinguishable from it by clinical, biochemical, and histologic findings.

Kennedy, A. E., Shklair, I. L., Hayashi, J. A., and Bahn, A. N.: Antibodies to Cariogenic Streptococci in Humans. *Archs. Oral Biol., 13:1275-1278, 1968.*

One possible mechanism for immunity to dental caries may be the inhibition or destruction of oral cariogenic bacteria by substances in the saliva, blood or oral tissues. Chief among these antibacterial substances are antibodies or immunoglobulins. The degree of immunity to cariogenic bacteria then may be dependent, in part, upon the concentration of antibodies specific for the cariogenic bacteria in the saliva, blood and oral tissues.

Smith, R. D. and Dienhardt, F.: Unique Cytoplasmic Membranes in Rous Sarcoma Virus-Induced Tumors of a Subhuman Primate. *J. Cell Biol., 37:819-823, 1968.*

Although the natural host of the Rous sarcoma virus (RSV) is the chicken, many strains of the virus have induced tumors in various mammals. Recently, a New World monkey, the marmoset (Saquinus sp.), was added to the list of animals susceptible to RSV oncogenesis. This report deals with a unique membranous cytoplasmic structure found in cells from two intracerebral and four subcutaneous tumors which developed following neonatal inoculation with the Schmidt-Ruppon strain of RSV.

Lindenbaum, Arthur and Kuettner, Klaus E.: Mucopolysaccharides and Mucoproteins of Calf Scapula. *Calc. Tiss. Res., 1:153-165, 1967.*

In the ossifying calf scapula the sharp division between cartilage and bone was found to allow collection of nearly unlimited quantities of both cartilage and bone in any desired stage of the transformation process. The mucopolysaccharides (MPS) of all zones consist mainly of galactosaminoglycans of the chondroitin sulfate A and C type. The highest concentration of MPS and mucoproteins (MP) was found in the 2 mm-wide cartilage zone adjacent to the bone. Significant differences among cartilage zones were observed in the quantity and nature of the extracted MP, with a rise in equivalent weight and a reduction in exchangeable sulfate toward the cartilage-bone junction. Removal of co-extracted serum proteins altered the MP values, presumably by making available functional groups which are covered up or occupied in the natural state. Microradiograms indicate regions of especially concentrated radiopaque mineral deposition in the vicinity of all chondrocyte nuclei and in a narrow region of cartilage immediately adjacent to the newly formed bone.

Brody, Lita, Webster, Mary C., and Kark, Robert M.: Identification of Elements of Urinary Sediment with Phase-Contrast Microscopy. *J.A.M.A., 206:1777-1781,* Nov. *1968.*

Because of the excellent visualization and definition of the urinary sediment, [in phase-contrast microscopy] greater accuracy can be achieved in reporting proportionate numbers of various casts. Also, clear photography is greatly enhanced, permitting record and teaching files. With phase-contrast, staining of the urinary sediment is usually unnecessary and adds very little to the clarity achieved.

In our laboratory we have found that the time required to teach physicians as well as technicians the elements of examining urinary sediments is greatly reduced. We have also found that when phase-contrast is used to study a large number of urines, less time is required than with bright-field microscopy. Therefore, it is our belief that phase-contrast microscopy should be used for routine microscopic study of urinary sediment of patients, both in the physician's office and in the hospital laboratory.

Apter, Julia: Some Problems in Visco-elasticity. In *Some Mathematical Models in Biology,* **ed. R. M. Thrall, J. A. Mortimer, K. R. Rebman, and F. R. Baum. Ann Arbor, Michigan, University of Michigan Press,** *1967, pp. A05.1-A05.8.*

Several Illustrative examples of visco-elasticity are presented with references.

Apter, Julia T. and Skaggs, Lester, S.: An Analogue Computer Solution to a Set of Non-linear Differential Equations of Motion of the Dog Aorta. I. The Effects of Hypertension, Aging and Blood Inflow Forcings on Pressure Curves. *Data Acquisition and Processing in Biology and Medicine, 5:1-13, 1968.*

One aim of this study was to produce pressure curves via a mathematical model of a dog aorta under normal, hypertensive and aging conditions. The pressure curves so generated with the aid of an analog computer solution of the model resemble real dog aortic pressure curves in general and also in several particulars: there is a dicrotic notch, a plateau and hump in diastole and both are less noticeable in hypertension. These similarities indicate that the model, besides being suitable for analyzing the behavior of the dog aorta *in vitro* is a reasonable starting point for investigating *in vivo* aortic pressure curves with the intention of improving the mathematical model still further.

Another aim was to demonstrate the non-linearities of the system and to show that these were amenable to analysis with a computer. Figure 10 and the table in the legend show where the non-linearities may be found. Since it is possible to force a circulatory system sinusoidally, it is logical to suppose that the real aortic system can be investigated for similar non-linearities, again with the aim of improving the model.

More than that, the computer solution made it possible to examine the effects of each parameter on model-generated curves in detail greatly exceeding that previously possible. For example, we were able to see the need for a distribution of n's rather than a single value. And if a distribution of n's is advisable, why not a distribution for a', a'' and even 1. Thus, these experiments on the model have indicated that a stochastic rather than a deterministic model is called for, thereby improving the model. What is more, we have used a lumped aorta when we know that dimensions and elastic and viscous constants vary down the aorta. Therefore, a distributed or at least a large number of contiguous, lumped systems to represent the aorta would be preferable and feasible with the computer. As with any good model, then, careful perusal of this one has led to improvements in it and to clarifications in the real system.

Apter, Julia T. and Graessley, W.: A Relation Between Positive Phase Shift and Elastic Modulus Enhancement of Smooth Muscle. *Experientia, 25:145-147, 1969.*

Smooth muscle, like insect flight muscle, develops a positive phase shift between an oscillating strain and the developed stress, indicating a release or production of energy. If the positive phase shift develops, an increase in elastic modulus also develops as well as a prolongation of *in vitro* viability of the smooth muscle. This phenomenon may prove useful in investigating the excitation-contraction coupling mechanisms in muscle. It may also prove useful as a simple and rapid means of testing the viability of muscular tissues in organs preserved for transplantation.

Douglas, Bruce L. and Greene, Howard J.: Reasons for Discrepancies in Reports of the Amount of Fluoride Present in a Source of Water. *J. Public Health Dentistry. 28: 49-51, 1968.*

The variations in the measured fluoride in the water naturally available to communities seems to depend primarily on the source. There are additional variables such as pollution of streams and lakes and differences in the construction, depth and location of wells. If a given community contains all of the types of wells noted in this report, it becomes unreasonable to generalize on a single figure for the entire community. Consistent data will be possible only when a central mixer distributes the entire supply of water, when controls or analyzers and automatic feeders are adjusted for all changes, when clean standardized bottles are used for all samples, and when accurate methods of analysis are used by competent analytical personnel.

Klawans, Arthur H.: Psychosomatic Disease in the Female. *Chicago Med., 72:211-215, March, 1969.*

From the viewpoint of the obstetrician-gynecologist the possibility of the occurrence of psychosomatic disease in the female exists from the time of puberty until the end of her life. The female is born as such and remains so during all of her years. The physiologic crises during her span of life are multiple, and each such crisis carries with it the danger of attendant psychic problems. Beginning with the time in life when feminine body characteristics become evident, through the adolescent period into the premarital years, through pregnancy, the pospartum time, menopause and old age, each step of the way has its bogies that can be responsible for symptoms on a purely psychogenic basis. It is our function to recognize that the phases of life tending to produce psychic problems are real, to prepare our patients for the possibility of upheavals and to help them face these critical periods with the least possible disruption of their lives.

Mörch, E. Trier: Pain Relief for Office Procedures. *Med. Clin. No. Amer., 52:173-181, Jan. 1968.*

Pain is one of the most common and most disturbing symptoms; in a great number of cases it can be relieved in the office. General anesthesia and generalized analgesia are best avoided unless the doctor and his staff are well trained and properly equipped.

Local analgesia by simple and peripheral techniques can be used safely in many conditions. The toxic doses of the analgesic and vasopressor drugs must be calculated to avoid toxic reactions.

Simon, Gerald, Rouser, George, Kritchevsky, Gene, Yamamoto, Akira, and Knudson, Alfred G., Jr.: Accumulation of a Glycerolphospholipid in Classical Niemann-Pick Disease. *Lipids. 3:287-290, May 1968.*

Accumulation of sphingomyelin in organs (1) and a deficiency of sphingomyelinase (2) have been established in classical (infantile) Niemann-Pick disease. We report here the accumulation of a glycerolphospholipid, tentatively identified as lysobisphosphatidic acid, in classical Niemann-Pick disease and some apparently related disorders.

Schwartz, Theodore B.: Who is a Diabetic? Editorial. *Ann. Int. Med., 69:161-163, July 1968.*

A mildly abnormal glucose tolerance test does *not* establish the diagnosis of diabetes—not even "chemical" or "asymptomatic" or "latent" or "preclinical" diabetes. Nor does a normal test provide any real assurance that a patient is free of diabetes and its consequences.

Zuber, Bert L. and Stark, Lawrence: Dynamical Characteristics of the Fusional Vergence Eye-Movement System. IEEE *Trans. Sys. Sci. Cyber., 4:72-79, March 1968.*

Experiments have been performed on the fusional vergence eye-movement mechanism in humans to provide a comparison with the established dynamical characteristics of the versional eye-movement system. Transient and frequency response experiments indicate that the fusional vergence system is not characterized by sampled data or refractory operation. When provided with a periodic input, this system may utilize prediction to reduce inherent phase lags. The gain of the system, although apparently unaffected by the predictive mechanism, is subject to input amplitude-dependent nonlinearities. Under conditions of artificially high loop gain, the system breaks into smooth sustained oscillations at a frequency predicted by frequency response data. The absence of a refractory period in the fusional vergence system is demonstrated by the system response to brief pulsatile stimulation. These results are discussed, emphasizing comparison with corresponding results from experiments on the versional system.

Smith, James, D., Ichinose, Lester Y., Masek, Gerald A., Watanabe, Takeshi, and Stark, Lawrence: Midbrain Single Units Correlating with Pupil Response to Light. *Science, 162:1302-1303, Dec. 1968.*

The consensual response of the pupil in the cat was driven by means of a light flux impinging on the contralateral retina. Spike trains recorded extracellularly from single units in the midbrain show correlation with the concurrently recorded pupil area. The temporal dynamics found confirm two earlier studies of single-unit responses and quantitative nerve stimulation. Both of these indicate that most of the 200-millisecond transport delay resides in the neuromuscular apparatus. Neurons whose activity correlated either with constriction or with dilatation phases of change in the pupil area were observed.

Stark, Lawrence, Arzbaecher, Robert, Agarwal, Gyan, Brodkey, Jerald, Hendry, Derek P., and O'Neill, Willim: Status of Research in Biomedical Engineering. IEEE *Trans. Sys. Sci. Cyber., BME-15:210-231, July: 1968.*

Bioengineering, biomedical engineering, or engineering for biology and medicine, is that field which deals with the interaction between the engineering sciences and biology and medicine. Because engineering is really two fields—*building*, using scientific principles, and *analysis* of complex mechanisms—bioengineering also is two fields: one concerned with building devices for biology and medicine, especially the development of instrumentation and data processing systems; and the other, the analysis of complex biological systems by means of the application of engineering science. At the present time, biomedical engineering is experiencing a rapid and vigorous developmental phase in many university, government, and industrial laboratories throughout the country, especially those that are in the forefront of scientific and engineering teaching and development.

This status report is divided into eight sections. The first four represent the classical engineering or building aspects of bioengineering. It is perhaps the area that most people think of primarily when biomedical engineering is mentioned. Section I deals with biomedical instrumentation, including both transducers for measurement and control, complex transducers, and systems of instrumentation. Section II discusses prosthetics, or the field of artificial organs. Section III, on Man-Machine Systems, includes the interaction of man with machines for working and the interaction of man with his environment so as to control material in the environment rather than pollute it. Section IV, on computers and information systems, differs from the previous ones in being less hardware-oriented and more information systems-oriented. It stresses the important impact of digital computers in all new technical fields.

The next three sections are related to the scientific, intellectual, and academic influence of bioengineering in the life sciences. Section V deals with classical engineering physics applied to such areas as cardiovascular hemodynamics and cardiac electrical field studies. Section VI, cybernetics or systems science, includes control, information theory, the analysis of physiological, biophysical, and biochemical systems with engineering conceptual methods, and bionics, which has to do with the use of biological principles in widening concepts of engineering design. Section VII, on education, deals with interactions between bioengineering and both engineering and biomedical curricula, as well as with bioengineering training programs, whose academic disciplinary status is still unresolved.

Finally, Section VIII is concerned with future goals of bioengineering both in terms of directions, problems, and probable ways in which needs may be met. It suggests certain foci of interest that may be helpful in planning efforts attempting to utilize limited resources effectively.

BYTERIAN·ST. LUKE'S HOSPITAL MEDICAL BULLETIN

PRESBYTERIAN-ST. LUKE'S HOSPITAL

MEDICAL BULLETIN

VOL. 8 NO. 4 OCTOBER 1969

The Effect of Previous Anti-parkinson Surgery on L-dopa Treatment

Dental Care in Presbyterian-St. Luke's Hospital

Organ Transplantation: A Review

Abstracts

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THE EFFECT OF PREVIOUS ANTI-PARKINSON SURGERY ON L-DOPA TREATMENT

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L-dopa (L-3, 4 dihydroxyphenylalanine) is now being tested in the treatment of parkinsonism in many centers throughout the world. In a preliminary analysis of the results obtained with this agent in 106 patients it appeared that patients who had previously undergone bilateral stereotaxic surgery for the relief of parkinsonism did not derive as much benefit from L-dopa as patients who had not undergone such surgical procedures.[1] The number of patients included in this and other reported series is small and the data is insufficient to analyze for the effect of prior surgery independent of other factors such as age, duration of disease, and severity of disease.[2,3,4] It is the purpose of this paper to evaluate the effect of previous surgery on the response to chronic L-dopa therapy by analyzing the results of such therapy in six matched pairs of patients.

METHODS AND RESULTS

All patients (120) treated with L-dopa were numbered consecutively, based on the date L-dopa therapy was initiated. Six of these patients had previously undergone bilateral stereotaxic neurosurgical procedures which included both pallidectomies and thalamotomies. In all cases surgery had been carried out at least two years previously. The disability of each patient was estimated according to the Northwestern disability scale.[5] This is a scale which grades the patient's disability in six separate categories. Each category (walking, dressing, eating, feeding, hygiene, and speech) is graded numerically, and the sum of the six is taken as the total disability.[1,5] In our study, the mate for each patient with surgery was chosen by taking the next patient on the list within five years of the same age and within five points in disability. The six pairs of patients are listed in Table I. The patients with previous surgery do not differ appreciably from those without surgery as far as age, disability, or duration of disease are concerned.

All patients were begun on L-dopa therapy as outlined previously and maintained on such therapy for at least two months.[1] At the end of this time the disability of each patient was again esti-

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TABLE I. INITIAL EVALUATION OF PATIENTS

Patient	Age	Duration of disease (Years)	Disability
Patients with previous bilateral surgery (S)			
1 S	62	15	36
2 S	74	13	38
3 S	66	15	39
4 S	63	11	26
5 S	74	5	28
6 S	68	13	31
Average	67.8	12.0	33.0
Patients without previous surgery (NS)			
1 NS	62	11	32
2 NS	76	12	40
3 NS	71	10	43
4 NS	68	11	28
5 NS	72	8	25
6 NS	69	9	31
Average	69.6	10.2	33.2

mated. Evaluation of the disabilities after two months of chronic oral L-dopa therapy is shown in Table II.

If prior stereotaxic surgery has no effect on the improvement brought about by L-dopa therapy both members of each matched pair should manifest similar improvement. The improvement in each individual was calculated by subtracting the disability after two months of L-dopa from the disability prior to institution of L-dopa. Each matched pair was compared by subtracting the improvement of the control or non-oper-

TABLE II. RESULTS OF LONG-TERM L-DOPA THERAPY

Patient Pairs S: Surgical NS: Non-surgical		Disability-rating on L-Dopa		Improvement over previous Disability		Improvement Difference S — NS
1.	S	33		3		−15
	NS		14		18	
2.	S	38		0		−16
	NS		24		16	
3.	S	9		30		+ 4
	NS		17		26	
4.	S	20		6		− 8
	NS		14		14	
5.	S	28		0		−18
	NS		7		18	
6.	S	28		3		−27
	NS		1		30	
Average		26.0	12.8	7	20.3	Total −80

ated patient from the improvement shown by the operated patient (see Table II). The sum of these differences would approximate zero if the matched pairs do not differ in relation to L-dopa therapy. The sum of differences is minus 80. This is significant at the 5% level ($.025 < p < .050$).

DISCUSSION AND CONCLUSIONS

From the data presented above, it appears that prior bilateral stereotaxic surgical procedures done for the relief of parkinsonism have an adverse effect on the subsequent response to chronic treatment with L-dopa. It has been shown that L-dopa is of significantly greater value in the treatment of parkinsonism than any of the medications now in general use. [1,3,4,6] It appears that this will soon be the treatment of choice for this disease.

The adverse effect of surgery on the subsequent response to L-dopa raises the question as to whether such surgery is warranted. Since surgery does not prevent progression of the disease to significant disability, it is our feeling that bilateral surgical procedures should not be carried out. All the patients in this series had severe disability. It is possible that previous surgery would not affect the outcome if L-dopa therapy were initiated earlier in the course of the disease.

Unilateral stereotaxic surgery does not appear to alter the individual response to long-term L-dopa therapy.[1] It is possible that unilateral surgery may have a place in the management of patients with unilateral disabling tremor. Even these patients should be given a trial of L-dopa, however, since at least one-half of such patients show at least a 50% amelioration of tremor.[1]

SUMMARY

Previous bilateral stereotaxic surgery done for the relief of parkinsonism was shown to be significantly associated with diminished responsiveness to L-dopa therapy. This was shown in patients matched for age, duration of disease and disability. It is suggested that such bilateral procedures should no longer be performed.

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DENTAL CARE IN PRESBYTERIAN-ST. LUKE'S HOSPITAL

Bruce L. Douglas

Total dental care is new at Presbyterian-St. Luke's Hospital. Although emergency services have been available for many years, other types of dental care have been limited. This has been primarily true because of the limitation of staff and the availability of that staff for only a relatively few hours of each working week.

Dental care is not new in the American hospital. In the East, such departments have been active for many years, administering to the dental needs of patients who are already in the hospital and to the emergency needs of out-patients, through dental clinics. These departments, customarily manned by internes, and often residents, are backed up by voluntary staffs of general practitioners and dental specialists.

A recent survey by the Council on Hospital Dental Service of the American Dental Association revealed that 2,721 hospitals out of a total of 6,721 reporting, or 42 percent, maintain dental departments.[1] About 30 percent, Presbyterian-St. Luke's among them, offer dental programs approved by the A.D.A.

The dental activity in the majority of these programs is restricted to oral surgery and emergency care. Only in the larger teaching hospitals, especially those with university affilitaions, are dental programs spanning the gamut of comprehensive dental services.

Dental Services as part of Comprehensive Health Care

Dental care properly forms one link in the chain of comprehensive health services. Without oral health care, a "comprehensive" health program is not really comprehensive, since dental disease is the most prevalent of all the physical ailments to which man is susceptible.

A hospital-oriented and hospital-based dental program is conceived on the premise that dental care should be a part of the total health program, and therefore the dentist is a member of the total health team. Dental care administered in an environment where other medical specialists are readily available can help bridge the gap between dental services and other health services needed by patients. It may be achieved through a formal relationship between doctors, such as group practice or joint hospital staff membership, or merely as a result of the close physical proximity of health practitioners to one another.

In many patients, dental disease accounts for one facet of their total health problem. A history of systemic disease such as diabetes, cardiovascular disease, blood dyscrasia, clotting disorder, epilepsy, or other disorders might affect either their oral cavities or dental treatment. Hence, consultation between the dentist and physician is in the best interest of these patients. Effective consultation is

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often very difficult to bring about when the patient's personal dentist and physician are inaccessible to each other. A patient with a recent history of cardiac surgery, one on diphenylhydantoin (Dilantin), an active diabetic, or even one with an abnormal fear of dental procedures, may well benefit from joint consultation before, during, or after dental treatment. In such a patient, dental treatment can no more be isolated from comprehensive health care than comprehensive care can exclude dental treatment.

Ambulatory Health Services in a Community Hospital

Today's community hospital is not only an institution to which sick people gravitate; it is also becoming a neighborhood center from which comprehensive health services radiate. Those services which emanate from the hospital are often directed at ambulatory out-patients. A recently published report of the American Hospital Associalion[2] emphasizes that the hospital's role is changing from a "concentration of beds with out-patient facilities attached" to an "ambulatory care center with in-patient beds attached." These changing concepts of hospital function lean strongly toward greater emphasis on those health care services which have been traditionally rendered in doctors' private offices but which presumably could be more effectively rendered in an environment where total health diagnostic and therapeutic facilities are readily available.

The A.H.A. report further states that "the core of the hospital may remain intensive patient care, but its major service to the community should be ambulatory care—including multiphasic screening, preventive services, diagnostic and treatment services, home care programs, health-related social services and a network of relationships with other health care programs." The report also recognizes that many people, especially the urban poor, are using hospitals as the primary source of medical care. *Almost all of these trends directly affect dental and oral health care, and point up the basic* raison d'etre *for dental services, on a comprehensive basis, in a community hospital environment.*

Our Hospital's Answer to the Need

In response to an obvious need, Presbyterian-St. Luke's Hospital has begun to enlarge its dental facility. At the center of its expansion program, the hospital has encouraged the development of a large group dental practice in its Professional Building, which adjoins the hospital proper. The dental office of the group functions actively twelve hours a day, five days a week, and eight hours on Saturdays. It is also available for the use of the dental house staff at night for the care of emergency patients. The hospital's dental internes receive a significant part of their training in the group office, as well as in the hospital proper.

The group-practice office has begun to take over a substantial part of the responsibilities previously shouldered by the hospital's Health Center. As a result, it assumes the responsibility for the care of large numbers of patients whose fees are paid by third parties.

The existence of the group facility in a building adjoining the hospital lightens the patient load on the hospital itself. Preventive dental services for pregnant women and well babies are handled effectively because of the close proximity of the office to the Health Center obstetric and pediatric clinics. In addition, the group office is prepared to handle all emergency dental needs for hospital employees and can keep many on the job who might otherwise have to leave the hospital premises to seek dental care (Table I).

The hospital's Professional Building includes the specialized practices of many other members of the hospital staff, and the dental group provides an oral health component to the comprehensive services otherwise available in that building.

TABLE I

SERVICES RENDERED IN 1969, THE FIRST YEAR OF TOTAL DENTAL CARE	
In-patient visits	2,910
Out-patient visits	16,040
Consultations from other services	1,255
Emergency patients	1,012
Health Center (clinic) patient visits	1,265
Operating room procedures	114
Hospital employee emergency visits	250
Preventive dental visits (in management of obstetric, pediatric, etc. patients)	408

Oral Surgery and Special Patient Care

Complete oral surgical services are available under the auspices of the hospital's dental department, supported by twenty-four-hour-a-day emergency coverage, in cooperation with other departments such as otolaryngology, plastic surgery, general surgery, medicine, and pediatrics. Facilities for oral surgical care are maintained in the hospital operating room and in the dental group office, where ambulatory general anesthesia services are also available.

With the cooperation of the Administration of the hospital and the Division of Surgery, complete dental care facilities have been made available for use in the operating room or at the bedside of those patients who need it. Handicapped and difficult children or adults can receive total dental care under general anesthesia, for example, with modern high-speed dental equipment; and cooperative arrangements have been developed for joint patient care between the dental department and other interested hospital departments, *e.g.*, medicine, pediatrics, and psychiatry.

The Hospital's Neighborhood Health Center

Presbyterian-St. Luke's Hospital has an Office of Economic Opportunity-sponsored neighborhood health center, which has a large dental program. The hospital dental department backs up the neighborhood health center dental department with specialized and in-hospital patient facilities.

SUMMARY

Recognizing its responsibilities for the total health care of its patients, Presbyterian-St. Luke's Hospital has begun to develop a broadly based dental and oral health care program. This program revolves essentially around a large dental group practice which is housed in the hospital's adjoining and attached Professional Building, thereby assuring dental and oral surgery coverage by general practitioners and specialists twelve hours a day. The dental house staff, reinforced by attending staff members, provides coverage on nights and weekends. The existence of the group dental practice has also made it possible for a large portion of the hospital's indigent community to acquire high-level private dental care as part of its total health services. Within the hospital, with the cooperation of other departments, the dental department provides care for in-patients, emergency care for patients with dental, jaw and facial injuries, and complete dental services for patients who require medical consultation or general anesthesia.

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REVIEW ARTICLE

ORGAN TRANSPLANTATION: PAST ACCOMPLISHMENTS, PRESENT STATUS AND FUTURE HOPES

William G. Manax

Immunological Basis of Transplantation

In the early 1940's Medawar and co-workers established that rejection of skin allografts was an immunological phenomenon.[1] They demonstrated that skin grafts were rejected after a relatively constant time interval ranging from 10 days to 2 weeks. This was characterized by vascularization of the graft (within 3 to 4 days) and progressive necrosis.

Rejection time was shortened, usually to less than a week, when mice were challenged with a second skin graft. It was the accelerated rejection of the second graft or "second set" response which was the initial clue that the destruction of allografts was brought about by a mechanism of active immunization.[2] This was further borne out by the delayed-hypersensitivity type of histological lesion which characterized the rejected skin grafts.

Histocompatibility Loci and Transplant Antigens

First, let us consider a background concerning the genetic basis of histocompatibility. From work with highly inbred mice, it has been established that such animals have at least fifteen histocompatibility loci. In other words, there are at least fifteen independent sites upon chromosomes where a gene controlling the expression on a transplant antigen can reside. These genetic loci are called "*histocompatibility loci.*" On a particular chromosome, one of several possible genes present in the genetic pool of the population may occupy a single locus. These genes are called alleles or allelic genes. Normally, somatic cells contain paired chromosomes and at particular sites on each of these, an allele occupies a specific locus.

The DNA of a gene occupying a given histocompatibility locus, or region, is encoded with specific genetic information which is eventually transmitted to the cell cytoplasm by the messenger RNA. RNA directs the cytoplasmic synthesis of proteins which are ultimately incorporated into the cell membrane. These proteins represent the antigens which are of cardinal importance in immune rejection. Therefore, the biologically effective units of transplant individuality are cell surface antigens derived from an inher-

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ited chemical blueprint residing in the genetic material of the histocompatibility loci.

Strong Versus Weak Loci

Early studies on transplant immunity indicated that histocompatibility loci were not necessarily of equal strength. In other words, the type of allograft immune response elicited by the different transplant antigens of a single tissue could be quite different. This resulted in the concept of "strong" and "weak" histocompatibility loci. In the mouse, the most important locus is called *Histocompatibility-2*, or the *H-2* locus; it is the controller of antigens which evoke strong allograft immunity. A difference between donor and recipient at the H-2 locus results in rapid graft destruction. Differences at other histocompatibility loci result in slower rejection. Weak loci however, have additive effects. Thus, when there is a difference between donor and recipient at a single weak locus, rejection is slow, but when several weak loci are incompatible, the rejection rate may be as vigorous as when there is incompatibility at a single strong locus.

The concept of strong and weak histocompatibility loci determining the rates of rejection is of major importance since the homologue of the H-2 locus has been identified in other species. In man, the single strong histocompatibility locus is referred to as HL-A.

Recipients For Whole Organ Transplants

The actual decision as to whether or not a patient should be a transplant recipient is usually not difficult. There must be terminal insufficiency in a vital organ that is irreversibly diseased or damaged. Ideally, as pointed out by Dr. David Hume, the recipient should have no serious infection, no generalized irreversible systemic disease, no serious emotional instability, and preferably, should be under fifty years of age. The question of equitable selection of patients for an organ transplant presents several problems. Opinions and methods vary from an attitude of "first come, first transplanted" to the organization of a committee of physicians and non-medical community leaders who together make decisions to ensure impartial consideration of the moral, ethical, financial, and sociological factors involved. Many physicians have challenged the concept that it is morally and ethically justifiable to attempt to prolong life by "unproved, seemingly heroic procedures using borrowed human organs." John P. Merrill of Boston has nicely responded to such an argument by his statement that, "Policy in the field of organ transplantation must be governed not only by the moral and ethical principles delineated, which apply to many fields of medical endeavor, but by prolonged experience of a well-motivated scientific team with a plan to obtain maximum information supplemented by a broadly based supporting laboratory program." At present, not all patients who might benefit from transplantation can be accepted, principally because of a lack of transplant facilities, and the financial and logistic problems related to a large scale organ transplant program.

Human Cadaver Organs

In kidney transplantation, the highest success rate has been with the healthy, blood-related, family donor who thus offers distinct logistic and immunological advantages. However, in the case of unpaired vital organs, such as with the heart, liver and pancreas, the allograft necessarily must be provided by suitable cadavers. Clinical and experimental studies show that these tissues, promptly salvaged and preserved, will function normally when allotransplanted. Further, the use of organs from cadavers circumvents many of the moral, social, and ethical problems encountered when tissues from a living donor are used for the purpose of transplantation.

Most cadaver organ donors are patients who have died from traumatic accidents, or from cardiac or neurosurgical procedures. Patients with widespread cancer, general bacterial sepsis, severe, uncontrollable hypertension, or local disease in the potential organ graft are unsuitable as donors after death. Still, some believe that the use of organs from persons dying from primary brain tumors, which rarely metastasize, are acceptable.

The suitability of a cadaver organ for transplantation can be determined with assurance only at the time of its surgical removal.[3] Also, in the preterminal state, during which time there are variable degrees of organ ischemia secondary to hypotension, measures must be instituted in order to protect these deprived tissues. In addition to ensuring adequate oxygenation by a mechanical respirator, sufficient blood pressure must be maintained to permit perfusion of the vital organs, and there should be a satisfactory urine output to indicate that this is occurring. The use of various protective measures to decrease metabolic activity and protect the all-important cellular lysosomes during this preterminal period should also be considered; these vary from generalized body hypothermia to the administration of metabolic inhibiting agents such as chlorpromazine, steroids, or phenoxybenzamine.[4]

Immediately after death, further means to protect the organs include local hypothermia (by surface cooling or *in situ* cold perfusion into the major artery) or extracorporeal circulation with a pump oxygenator system. After an organ is removed from the cadaver donor, it can be kept viable temporarily by means of hypothermia and hyperbaric oxygenation.[5] Successful organ preservation in vitro permits the recipient to be fully prepared for reception of the graft without undue rush. In the laboratory, storage by hypothermia and hyperbaric oxygen will keep various canine organs viable in vitro for periods up to 3 days. Dr. Thomas Starzl and co-workers in Denver have applied this preservation method clinically for storage of livers from cadavers before allotransplantation. To date, this group has transplanted livers in 11 humans, and at the time of this writing, 2 of these patients are still alive. Also, Starzl has reported successful in vitro preservation of canine livers for 24 hours and longer with satisfactory hepatic function after orthotopic transplantation in dogs.[6]

To enlarge the scope of transplantation programs, organ banks will obviously be required, similar to banks at present for bone, skin and arteries. But it is likely that only when the various problems attending freezing and thawing of whole solid organs are solved will living organ banks become a reality. Another possible avenue of storage is the transplantation of human cadaver organs into subhuman primates such as the baboon; an organ could then be stored "in vivo" until required clinically. However, this will require more knowledge of immunosuppression than is currently available.

Donor-Recipient Matching

Immediate allograft failure from intrarenal red blood cell agglutination can be prevented by matching the major blood groups of donor and recipient. Major ABO blood group incompatibility is the most frequent mismatch. The Kidney Transplant Registry reports that about 48% of ABO-compatible donor kidneys function after allotransplantation, whereas only 15% of ABO-incompatible donor organs have survived. While major ABO blood group compatibility is important in donor selection, minor blood group incompatibilities, or Rh factors, generally appear to be of little practical significance in allotransplantation. On the other hand, blood group matching cannot be considered equivalent to genetic histocompatibility since red blood cells contain few transplantation antigens.

Several tissue matching methods have now been devised. These methods include skin tests, lymphocyte transfer tests (in vivo), leukocyte typing or agglutination (in vitro), and tissue cultures of lymphocytes. Dr. Paul Terasaki has developed a method of leukocyte typing in which donor and recipient lymphocytes are tested against standard antisera. Similar responses to these antisera from both donor and recipient lymphocytes suggest greater likelihood of histocompatibility. When the Terasaki test indicates a good match between donor and recipient, the number and intensity of graft rejections are reduced and the opportunity for long-term survival is significantly increased. In contrast to the facility and careful selection of kidneys thus possible from living donors, the use of cadaver donors necessitates a pool of potential recipients with various blood types who must be readily available to receive kidneys at any time from suitable cadavers. Because current histocompatibility tests require at least 4 hours to perform, these tests are only of retrospective value when cadaver tissues are used in many cases.

Thus, major research concerns are the development of rapid, reliable tissue typing techniques for selection of the proper recipient and the improvement of methods for organ preservation.

Allograft Rejection

There are obviously many reasons why a transplanted organ may fail to function, but the principal problem is rejection of the graft by the host. Earlier in this communication we saw that graft rejection is a hypersensitive, immunological reaction of the delayed variety caused by contact between antigenic tissue cells of the donor introduced into the recipient's body, and sensitized lymphoid cells. In reality, the precise mechanism of the rejection phenomenon is not clear, although the vasculature of the transplant seems to bear the principal effects of the antigen-antibody reaction. A transplanted organ undergoing rejection develops a characteristic infiltrate of lymphocytes, plasma cells, and polymorphonuclear leukocytes in its interstitial and perivascular structures. When acute rejection occurs, vascular endothelial damage is evident with ensuing endothelial proliferation eventually leading to the classic vascular occlusion of chronic rejection.[7] It is likely that the majority of allografted organs in humans display at least one episode of threatened rejection in varying degrees of intensity.

Clinically, threatened rejection of an allograft is suspected when the patient develops progressive signs of impaired graft function in association with fever, leukocytosis, and a tender, edematous transplant. Threatened rejection often can be aborted with increased doses of steroids or the administration of actinomycin-C and local irradiation to the graft. But acute rejection appears abruptly and may irreversibly damage an allograft despite all forms of therapy. By definition, acute rejection occurs within the first 4 months after transplantation. Rejection of an allograft after this period is rarely acute, and is manifested clinically by a slow but progressive loss of graft function.

Reversal of a rejection crisis necessitates intensified immunosuppressive therapy which in itself may represent a threat as great as rejection. Intense immunosuppression increases the patient's susceptibility to infection by fungi, viruses, or bacteria. It is thought that relatively few patients die primarily from a rejection reaction. Rather, they die from infection that follows concentrated treatment with immunosuppressive agents. Overall, about 55% of transplant deaths result from infection of one sort or another.

Methods to Prevent Allograft Rejection

More often than not, it is after the surgical transplant procedure that the real problem begins. That problem of course: rejection.

The treatment of allograft rejection changes almost from day to day. Part scientific, part empirical, part investigative, all these efforts ultimately aim to induce a state of specific immunological tolerance by which the host can expect a long-term survival with a functioning—albeit foreign—organ. Perfect tissue matching and complete mutual histocompatibility is the ideal and, hopefully, this will become a reality in the near future. However, at the present time we are left with immunosuppression, because perfect matching techniques are not available. Despite the numerous problems accompanying the use of immunosuppressive therapy, inroads are being made here by the use of steroids, antimetabolites, radiation, and other techniques.

Uphoff, in 1958, showed that methotrexate could modify the rejection phenomenon,[7] and shortly after this, Schwartz introduced 6-mercaptopurine.[8] Yet, neither of these drugs was wholly satisfactory in the human. The outlook for drug suppression of rejection seemed quite dismal till the introduction of azathioprine (Imuran). This drug seemed to depress the allograft rejection without inordinately depressing bone marrow and the overall immune phenomona which protect us against infection. Yet, even with this drug it is possible to overdose patients, particularly in the early postoperative period when the renal allograft is often oliguric. Still, however, it is the pharmacological agent which has allowed us to obtain clinical successes in renal transplantation in more than just an occasional patient. Most transplant groups now use azathioprine in combination with various dosages of natural or synthetic glucocorticosteroids. At the time of rejection crises, steroids are given in massive doses, most commonly prednisone in the dosage of 2 to 4 mg/per/kg of body weight. Occasionally also, during acute rejection crises which do not respond to elevated doses of azathioprine or steroids, actinomycin-C which is a potent cytotoxic agent may be given in a dose of 1 mcg/per/kg of body weight.

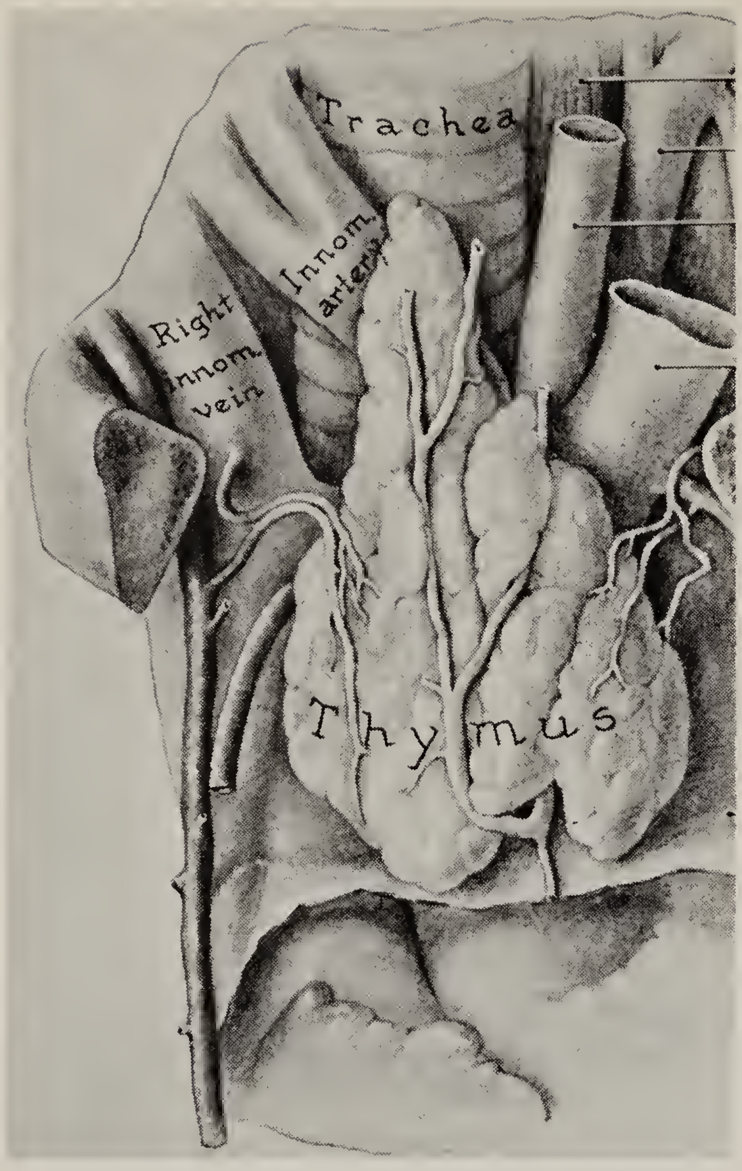
Irradiation given with high-voltage X-ray machines or from cobalt, has been employed to suppress rejection. Initially, whole body irradiation was given, but with the exception of a few patients of Hamburger[9] who survived, all other recipients so treated usually died from overwhelming infection.

More recently, cobalt irradiation has been used in a limited manner. During a rejection crisis, only the renal allograft is irradiated in situ with 150 to 200r daily, or every other day for 3 doses. The rationale of this therapy is to destroy the small lymphocytes of the recipient which invade the allograft during rejection.

The small lymphocytes deserve further mention. Most small lymphocytes pass, at one time or another, through the thoracic duct, and the majority of these are not newly formed cells but have a life span measured in months or years. Gowans[9] in 1965 noted that the number of small lymphocytes in the dog can be depleted by draining the thoracic duct lymph through an external fistula. As a result, he found that the survival of skin allografts can be prolonged. Such thoracic duct fistulas have now been made in a variety of animals and in man to decrease the number of circulating small lymphocytes and consequently blunt the intensity of allograft rejection. So far in man, the results are indeterminate, but are promising and deserve continuing investigation.

Initially in clinical renal transplantation, splenectomy was usually carried out at the time of bilateral nephrectomy in the recipient. This was done because of the belief that splenectomized patients could tolerate higher doses of azathioprine without bone marrow depression. Recent information now contradicts this view, and in both animals and in man there seems to be no significant difference in the tolerance to azathioprine with or without the spleen. Consequently, splenectomy has been abandoned by most groups.

In recent years, Good[10] in this country, and Miller[11] in England, have greatly increased our knowledge of functions of the thymus gland (Fig. 1) which has puzzled so many for so long. This basic information on the function of the thymus has come at a propitious time, for it appears that the thymus is a master gland for the development of the immunological



FUNCTION OF THYMUS

1. **Populate peripheral lymph tissue with small lymphocytes**
2. **Maturation of lymphocytes via hormonal effect**

Fig. 1.—Recent information shedding some light on thymic function has provided valuable information on better understanding of transplant immunity.

uniqueness which characterizes each of us. Thymic function is apparently largely completed during intrauterine and early neonatal life. This information may explain the confusion over the atrophied thymus of adult life. From what is known at present through the pioneer research efforts of Burnett, Medawar, Miller, and Good, the function of the thymus can be tentatively described in the following way. During intrauterine life, stem cells from the bone marrow develop into large lymphocytes which travel to the thymus and to the peripheral lymph tissue in the intestine and appendix. In the thymus, the large lymphocyte changes into a small lymphocyte which then migrates out to the peripheral lymph nodes. Once in this peripheral location, the small lymphocyte is probably aided further in its maturation by a hormone secreted by the thymus. Other large lymphocytes travel to the lymph tissue in the bursa of Fabricius in lower animals, or to the lymph tissue of the appendix and terminal ileum of higher mammals, and there, apparently differentiate into the plasma cell series. It is thought that the thymus is primarily responsible for the development of cellular antibodies through the small lymphocyte and is concerned with the development of cellular immunological competence, while the distal lymph tissue in the terminal ileum, appendix , or bursa of Fabricius is more concerned with the development of humoral competence. This separation of cellular and humoral immunological competence may be the key point in the future of clinical transplantation. Perhaps we can hope eventually to produce immunological tolerance to an allograft without necessarily damaging the recipient's ability to react against infection and the other vicissitudes of daily living.

Clinically, only Starzl of Denver has had any extensive experience with thymectomy in conjunction with renal allotransplantation. After doing a few thymectomies along with renal transplantation a few years ago, Starzl abandoned thymectomy because of the high morbidity associated with it. Yet recently, he has surveyed the results and found that patients with thymectomies have probably done somewhat better than patients with an intact thymus.[12] Starzl and others are now undertaking new studies in this area.

Future Horizons

Organ Freezing

The monumental discovery of the protective effects of glycerol by Parkes and Polge at Cambridge in 1949,[13] stimulated the development of methods for freezing bull spermatozoa. Now a significant portion of dairy cattle in the U.S.A. are conceived by artificial insemination using preserved sperm. Glycerol also has been used to allow freezing of whole blood. After preservation at low temperatures for a year or longer, more than 50% of the erythrocytes are viable after thawing. Such accomplishments have caused renewed interest in the search for other protective or cryophylactic agents in addition to glycerol. There are now many such agents known to protect cells against the adverse effects of freezing and thawing, but despite this, there are still no conclusive reports of successful freezing and thawing of organs, with subsequent satisfactory function. The basic problem does not lie in the need for new knowledge. If we could uniformly saturate the billions of cells of each organ with a cryophylactic agent before freezing, with the same facility that a thin film of blood or spermatozoa can be saturated, we should be successful in freezing and thawing whole organs. It is likely that frozen cadaver organs will be the principal source of organs for transplantation in man before the turn of the century.

Artificial Organs

Already it has been amply shown that temporary internal or external mechanical devices may successfully serve as "tiding-over" measures for support of a damaged organ undergoing repair or for an engrafted organ which has not resumed maximal effective function. The so-called artificial kidney and heart-lung machine readily attest to this concept. Although replacement of a diseased organ such as the heart by an artificial pump within the body may hold future promise, it is unlikely that replacement of vital organs such as the kidney, liver, or endocrine glands by mechanical devices within the body will be realized in this century because of the anatomical and functional complexity of these tissues. In the case of renal allotransplantation versus chronic hemodialysis, there is no doubt that the patient with a successful kidney transplant feels better and has far fewer problems than the patient who is maintained by periodic dialysis. Patients who must undergo chronic hemodialysis for maintenance of life are forced to tolerate an existence complicated by restrictions of diet and activity, while infection, anemia, and hypertension always pose serious threats. In contrast, when a renal transplant is successful, the patient may lead a relatively unrestricted life and enjoy a feeling of well-being.

Heterografts may become clinically practical in the future, especially when improvements in methods to thwart rejection crises are a reality. Successful heterografting would circumvent the complicated logistic problem in organ transplantation, not to mention the moral and ethical issues concerned.

Unexpected Benefits Of Transplant Research

Transplantation is an interdisciplinary field. It requires the talents of pediatricians, internists, surgeons, immunologists, microbiologists, physiologists, and biochemists, in addition to numerous paramedical specialists. Conversely, over the past ten years, transplantation has taught each of these specialties a great deal about its own field of interest. The transplantation of a normal organ into a diseased individual is a most powerful experimental tool for determining whether local or general systemic factors are involved in the pathogenesis of a given disease process. A disease which is primarily an affliction of the kidney, for example, and whose etiology and pathogenesis are entirely in the organ itself, is cured by a kidney transplant. On the other hand, a disease which is systemic and which affects the kidney only secondarily would be expected also to affect the kidney transplant. Consider some examples to illustrate this concept.

The first type of disease is illustrated by cystinosis. This is an inborn error of the metabolism characterized by deposition of cystine throughout the reticuloendothelial system and in numerous organs—especially the kidney. It commonly causes death during childhood due to renal failure. The damage however, is not primarily wrought by extrarenal factors damaging the kidney. Therefore, one would predict that a kidney transplant would restore renal function and also be resistant to the development of the cystinosis lesion. This reasoning has been borne out in a single case of a kidney transplant in a child suffering from this disorder.

On the other hand, consider glomerulonephritis. Experience with identical twins has taught nephrologists a great deal about this particular disease. Most notably, it has been shown that the rate of recurrence in the transplant patient is high. This indicates that the pathogenesis of glomerulonephritis involves the continuation of host responses long after the onset of the disease. But transplant studies have also underscored a certain few of the analogies that may exist between glomerulonephritis and the immune rejection process. And the implication is clear; perhaps immunosuppression of the type used in transplantation is of value in halting the progression of the glomerulonephritic lesion. It may become a major therapeutic tool in a wide variety of so-called autoimmune diseases.

Anephric patients, maintained on pretransplant hemodialysis, have provided some insight into the mechanism of renal hypertension and the role of the humoral substances synthesized or metabolized in the kidney. As an example, even though the kidney is a major site for the production of erythropoietin, normal erythropoiesis can continue in the absence of renal tissue. Renal hypertension yields rapidly to hemodialysis following bilateral nephrectomy, while hypertension from other causes responds more slowly.

Since the kidney is the major source of renin production, serum renin levels decrease, as expected, following total nephrectomy. On the other hand, aldosterone (whose output from the adrenal gland is enhanced by the conversion of renin to angiotensin) remains at normal levels. During volume depression, however, there is no increase in aldosterone response, indicating that the increased adrenal output of aldostrone under these circumstances is kidney-mediated.

Transplantation investigation has also contributed to our understanding of the mysterious nature of "aging" for which biochemists still offer no plausible explanation. The aging process has been overcome by repeatedly transplanting tissue from old animals into young ones. The same graft can thus be re-transplanted perpetually and does not appear to age at the rate of the original donor. These experiments seem to be duplicated in parent-child kidney transplantations. Whereas the renal function of the parental donor decreases with aging, that of the child who lives entirely on the donated kidney, seems to remain stable. Therefore, it appears that there are many complicated biological and pathological factors to be understood in order to know what is really meant by the "life" and "aging" of a vital organ.

CONCLUSION

The greatest advances in organ transplantation have been in surgical technique. Our basic research in immunology and in organ preservation, though less impressive and less well established, has made remarkable recent strides, and current work in these areas is indeed encouraging. Nevertheless, resolution of these medical problems will leave us with still other pressing dilemmas. The economics of transplantation, rarely mentioned, are important. Even in the absence of professional fees, for example, it is almost inconceivable to envisage current hospital, drug, and laboratory costs less than $10,000 per patient, and in particular situations, the monetary burden may climb to as high as $40,000 or $50,000. If we are soon able to transplant at will, how shall we make the choice of patient? The hope is that all who need an organ transplant will have it done. Yet, this does not seem likely as transplantation procedures become more complicated.

Ethical problems concerned with removing organs from living donors for transplantation may soon be nonexistent in that we are using more cadaver organs. But, in this connection, when is a patient dead? When his heart stops? When the waves on the electroencephalogram are flat? Or, is it when the potential donor no longer can sustain respirations without a respirator? The answers to these questions are important, because the earlier that organs are removed from cadavers and transferred to living recipients, the more likely the success of the transplant procedure. The National Academy of Medicine of France has declared that a man whose heart is still beating may be ruled dead if it is decided that the brain will not resume control of vital functions. This decision sets a precedent because it allows the possibility of removing a healthy, beating heart from a person whose brain is irrevocably damaged. Overriding all these questions is the spectre of over-zealous interpretation of such decisions so that errors could be made and vital organs removed from donors who might still have a chance of recovery. Finally, and an even more pressing problem, may be the very success of these procedures. If transplantation becomes medically and economically feasible on a wide scale with a low morbidity and mortality, what will become of those of us who get new visceral organs but retain the same senescent brain? Replacement of the brain will be the last and most difficult of all transplantation procedures. There may be many of us who possess no desire to live beyond the Biblical "three score and ten," if living is only existing without friends or loved ones.

It is essential to end on this somewhat pessimistic note because of the penchant of all of us to ignore, or gloss over, the non-medical problems associated with our profession. Such an attitude, certainly in the area of transplantation, is no longer acceptable and is incongruent with medical progress.

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ABSTRACTS

OF PUBLICATIONS BY THE STAFF

Ryan, Will G., Schwartz, Theodore B., and Perlia, Charles P.: Effects of Mithramycin on Paget's Disease of Bone. *Ann. Intern. Med. 70: 549-557*, Mar. *1969.*

Administration of mithramycin (25 ug/kg/day for 10 days) to two patients with advanced Paget's disease of bone resulted in clearcut beneficial effects. The patients' symptoms were ameliorated. Serum calcium and phosphate levels were lowered during therapy but promptly returned to normal afterward. Markedly elevated serum alkaline phosphatase levels were reduced considerably and have remained relatively low after therapy. Urinary calcium and phosphate levels were also markedly reduced during therapy, and negative calcium and phosphate balances became positive. Markedly elevated urinary hydroxyproline levels were reduced to less than one-fourth of control values. Bone accretion rates were significantly decreased.

These observations suggest that mithramycin administration diminished the elevated osteoclastic and compensatory osteoblastic activity characteristic of Paget's disease, and this agent may have therapeutic value in patients in whom there is wide dissemination of the pathologic process.

Manax, William G. and Lillehei, Richard C.: Transplantation of the Intestine. In: Diseases of the Colon and Rectum, ed. Robert Turrell, Philadelphia, W. B. Saunders, *1969, pp. 1310-1325.*

At the turn of this century, interest in organ grafting focused almost entirely on technical problems of organ replacement, especially vascular anastomoses. But the field has flourished so rapidly over the past two decades that it is best encompassed now by the term *transplantation biology*, which broadly includes a wide spectrum of subjects including genetics, immunology, embryology, physiology and other disciplines. The bulk of transplant effort has focused on the kidney, both in humans and in experimental animals. But over the years we have shown in the laboratory that the intestine is just as adaptable to allotransplantation as is the kidney. This communication has been designed to relate the more pertinent experimental and clinical developments relative to intestinal transplants. Experience to date indicates that not only are such transplants technically feasible, but that they are no more antigenic than are other tissues. Probably the main obstacle to developing intestinal transplants as a practical clinical endeavor has been the lack of a suitable experimental model. This obstacle has now been surmounted, and it is likely that hundreds of intestinal allografts in humans will be carried out in the next two decades.

Henson, Donald E., Najafi, Hassan, Callaghan, Robert, Coogan, Philip, Julian, Ormand C., and Eisenstein, Reuben: Myocardial Lesions Following Open Heart Surgery. *Arch. Path., 88: 423-430, Oct., 1969.*

Autopsies on all patients having open heart surgery during 1965 through 1967 were reviewed. Of 71 cases, 60 showed histological changes in the myocardium. The first change was the formation of contraction bands in the myocardial fibers, usually in the inner two-thirds of the left ventricular myocardium. Next, intersitial hemorrhage occurred in areas where contraction bands were present followed by coagulative necrosis. Occasionally, damaged myocardial fibers along the border of the hemorrhagic necrosis became calcified. Later, the hemorrhagic necrotic area was replaced by granulation tissue and fibroblasts laying down collagen. Cause of the lesions is unclear, although it may be related to inadequate perfusion of the subendocardial myocardium.

Balagot, R. C. and Bandelin, V. R.: Comparative Evaluation of some DC Cardiac Defibrillators. *Amer. Heart J., 77: 489-497, April, 1969.*

1. Four commercially available defibrillators were subjected to extensive tests of electrical characteristics.
2. Three of the defibrillators evaluated were found to deliver substantially less energy (20 per cent to 78 per cent less) than indicated by the energy selector controls, when discharged to a range of resistance usually encountered clinically.
3. The energy delivered by all LC circuit defibrillators varied as a function of body resistance. Unless calibrated in *delivered energy* rather than stored energy, the output may vary widely among defibrillators of different manufacturers.
4. All units tested had a characteristic internal loss of energy, which was superimposed on the loss due to mismatch with body resistance.
5. The single LC defibrillators, with waveforms of three or four milliseconds in duration, achieved extreme peak voltage and current amplitudes at high energy level selection. These amplitudes had previously been shown to be deleterious.
6. Since resistance measurements are generally not available to the clinician, and conversion tables relating stored energy to delivered energy would be awkward at best, some standardization of the outputs of defibrillators is indicated. It is suggested that calibration of the energy setting to indicate *delivered energy* to a 75 ohm load be adopted generally.

Fried, Walter, Rishpon-Meyerstein, Naomi, and Gurney, Clifford C.: The Effect of Testosterone on Erythropoiesis of WWv Mice. *J. Lab. Clin. Med., 70: 813-819, 1968.*

Testosterone increases the hematocrit, the hemoglobin concentration, and the red cell mass of WWv mice. It also raises the plasma erythropoietin level to greater than twice the pretreatment value, but doesn't correct the defect in the colonizing ability of WWv bone marrow cells. We suggest that these findings are compatible with the concept that the stem cells of WWv mice are defective in such a way that they can respond to erythropoietin only if it is present in very large amounts. Testosterone is capable of further increasing the plasma erythropoietin content to a level capable of stimulating these refractory cells.

Fried, W., Weisman, M., Martinson, D., and Gurney, C. W.: The Effect of Hypoxia on Colony-Forming Units in Bone Marrow. *J. Lab. Clin. Med., 71: 422-428, 1968.*

Prolonged exposure to hypoxia does not result in a decrease in the number of colony-forming units in a leg. The colony-forming units do, however, become significantly more sensitive to the damaging effects of X-irradiation after 3 days of hypoxia. We suggest that elevated erythropoietin levels result in an increased rate of erythorpoiesis. This, in turn, either directly or indirectly, stimulates multipotential stem cells to differentiate. To prevent a decrease in the pool of undifferentiated cells, the remaining ones are stimulated to proliferate more rapidly. Rapidly proliferating cells are, in turn, more radiosensitive.

Krantz, Sanford B. and Fried, Walter: In Vitro Behavior of Stem Cells. *J. Lab. Clin. Med., 72: 157-164, 1968.*

An in vitro method has been adapted for mouse spleen cells which permits the measurement of the effect of erythropoietin on heme synthesis and on colony-forming ability. In this system erythropoietin markedly increases the rate of hemoglobin synthesis indicating increased development of erythropoietin responsive cells into erythroblasts, but does not affect the total number of colony-forming units remaining in culture. The amount of erythropoiesis in the spleen colonies of irradiated mice is also unchanged after the colony-forming units are treated with erythropoietin in vitro. The effect of erythropoietin on hemoglobin synthesis cannot be demonstrated after 23 hours of incubation despite the presence of an unaltered number of colony-forming units at this time. Possible reasons for these results are discussed.

Kilbridge, Thomas and Fried, Walter: Response of Mice with a Decreased Number of Hematopoietic Stem Cells to Doses of Erythropoietin. *J. Lab. Clin. Med., 72: 85-92, July, 1968.*

Irradiated mice respond to any given dose of erythropoietin in a manner which is inversely proportional to the amount of radiation. They, however, retain the ability to respond to larger amounts of erythropoietin according to a dose response curve which is similar in shape to that in nonirradiated mice. Mice exposed to X-irradiation with a leg shielded have a small, yet undamaged, hematopoietic stem cell compartment. They also respond less well to any given dose of erythropoietin than do nonirradiated mice, and thay have a dose response curve which is similar in shape to that of nonirradiated mice. Accordingly, it is probable that this alteration in response to erythropoietin is a reflection of a decrease in the size of the stem cell compartment and is not peculiar to a specific type of radiation-induced cellular damage. These results are in accord with the following explanation: As the number of remaining stem cells becomes smaller, the chances decrease of any one cell coming into contact with sufficient erythropoietin molecules to trigger differentiation. Under these circumstances, erythropoiesis can be enhanced by increasing either the plasma erythropoietin titer, or the number of erythropoietin responsive cells, or both. We suggest that this model may be applicable to understanding the mechanism of anemia in some patients with high plasma erythropoietin levels, who respond to agents which further increase this titer.

Friedberg, Stanton A., Eisenstein, Reuben, and Wallner, Linden J.: Giant Cell Lesions Involving the Nasal Accessory Sinuses. *Laryngoscope, 79: 763-776, May 1969.*

The case histories of five patients with tumor-like growths involving the nasal accessory sinuses are presented. In each instance the original pathologic diagnosis was benign giant cell tumor. A critical reappraisal reveals four patients with giant cell reparative granuloma and only one with a probable giant cell tumor. Distinction between the two types of lesions is becoming increasingly well recognized.

The histologic differentiation between giant cell tumor and giant cell reparative granuloma can be extremely difficult, and the final diagnosis may depend upon the clinical course of the patient.

The brown tumor of hyperparathyroidism is histologically similar to the giant cell lesions under consideration and hyperparathyroidism should always be considered as a diagnostic possibility.

Local excision is usually effective in dealing with giant cell reparative granulomas. Irradiation is not recommended because of the long-term risks involved in its usage for benign conditions.

If a definitive diagnosis of giant cell tumor can be established, wide surgical extirpation is the treatment of choice. Irradiation is not advocated at the present time.

Holmes, Albert W., Wolfe, Lauren, Rosenblate, Howard, Deinhardt, Friedrich: Hepatitis in Marmosets: Induction of Disease with Coded Specimens from a Human Volunteer Study. *Science. 165: 816-817, Aug. 1969.*

Marmosets inoculated with plasma from three early acute hepatitis patients developed hepatitis 30 to 40 days later. Other groups of marmosets receiving preinfection plasmas from the same patients showed no evidence of hepatitis in this experiment. It is, therefore, most probable that hepatitis in marmosets represented transmission of human disease rather than activation of latent "marmoset hepatitis."

Eisenstein, Reuben, Battifora, Hector, Ellis, Hellen, Rosato, Jacquelyn, and Hobart, John: Effects of Sucrose and Dactinomycin on Hepatic Acid Hydrolases. *Arch. Path. 87: 514-520, May 1969.*

When rats are injected with 50% sucrose, enhanced pinocytic activity, altered intracellular distribution of acid hydrolases accompanied by enhanced liver activity, and increased urinary excretion of acid hydrolases are seen. In proper dosage, dactinomycin induced enhanced activity of three lysosomal enzymes in the liver when large volumes of hypertonic sucrose or 0.9% sodium chloride were also injected. Without these large volumes of fluid, only the activity of β-glucuronidase was increased. A possible explanation of these data is that synthesis of lysosomal enzymes is governed by a genetic repressor, although other interpretations are more likely. Perhaps the most acceptable one is that they are due to the relative stability of lysosomal enzymes compared to that of some other liver proteins with a resultant increase in their concentration. In conjunction with previous work, however, the data indicate that lysosomes can form in the presence of dactinomycin.

Henson, Donald: Cytomegalovirus Hepatitis in an Adult. An Autopsy Report. *Arch. Path., 88: 199-204, Aug. 1969.*

A young woman with juvenile onset diabetes mellitus was found on histologic study of autopsy material to have disseminated cytomegalovirus (CMV) infection. Intranuclear and cytoplasmic inclusions characteristic of CMV infection were present in most of the visceral organs. In the liver, there were intranuclear inclusions in hepatic cells, focal necrosis, and a hepatitis which was attributed to CMV. This case supports published virologic evidence that, in addition to young infants, CMV can cause a hepatitis in adults.

Ey, Richard C., Hughes, William F., Bloome, Michael A., and Tallman, Carter B.: Prevention of Corneal Vascularization. *Amer. J. Ophthal. 66: 1118-1131, Dec. 1968.*

Methods to combat vascular invasion of the cornea include severing the vessels at the corneoscleral limbus surgically or by cauterization, corticosteroids, beta radiation, and thio-TEPA. Many of the practical details and comparative value of such treatemnts are uncertain. For this reason various methods to inhibit the ingrowth of vessels were compared in rabbit corneas burned by the intracorneal injection of sodium hydroxide. A total of 324 eyes were used in these experiments. Final evaluation of results was made from examination of Kodachrome photographs taken at weekly intervals. The most effective forms of treatment were subconjunctival injection of a repository form of methyl prednisolone, beta radiation, and thio-TEPA. Complications with these forms of treatment were also considered.

Hughes, William F.: Treatment of Herpes Simplex Keratitis: A Review. *Amer. J. Ophthal. 67: 313-328, Mar., 1969.*

About two thirds of dendritic ulcers respond to instillations of antimetabolites such as idoxuridine (IDU), such treatment being especially valuable for children and for geographic ulcers. If a good response is obtained after five days, treatment should be continued for a total of two weeks. If not, cauterization and removal of the infected and adjacent epithelium should be performed. This latter well-established treatment has certain advantages as a primary treatment, especially in unreliable patients. The complications of IDU therapy are relatively infrequent. Local administration of corticosteroids is contraindicated for dendritic ulcer.

Stromal herpes keratitis requires restraint in treatment and patience on the part of the victim. Antimetabolites are ineffective. Cautious administration of corticosteroids is warranted only for severe cases with prolonged keratitis, relatively dense infiltration, early vascularization, and intense iridocyclitis. Unresponsive cases, especially those with deep ulceration and a poor visual prognosis, may require either a lamellar or penetrating corneal transplant.

The herpes virus in humans evidently can penetrate the stroma, remain there after apparent healing and produce a direct infection of the iris. Local administration of corticosteroids can increase replication of the virus and spread of infection.

Clasen, Raymond A., Pandolfi, Sylvia, Russell, James, Stuart, Duncan, and Hass, George M.: Hypothermia and Hypotension in Experimental Cerebral Edema. *Arch. Neurol. 19: 472-486, Nov. 1968.*

The edema associated with cerebral lesions produced by focal freezing in monkeys killed nine hours after injury was studied by quantitative chemical methods. This involved the measurement of hemispheric water, potassium, sodium, chloride and iron as well as the uptake of intravenously administered Evans blue and iodinated I_{131} serum albumin (Risa). The damaged hemisphere showed a significant uptake of all factors together with a decrease in tissue potassium. A correlative analysis of these data further suggested binding of sodium by the necrotic lesion, of chloride by the edematous tissue, and of dye by both.

The brains of animals subjected to sustained systemic hypothermia of 25° applied after injury showed diminished uptake of water and iodinated I_{131} serum albumin together with diminished binding of dye when compared to the controls. As judged by water increments, the degree of edema was reduced by about one fifth. There was no evidence of chloride binding and the total tissue sodium did not appear to be involved in this suppression. Supression of water uptake in the grossly normal portion of the damaged hemisphere was also demonstrated.

The brains of animals subjected to sustained systemic hypotension of about 70 mm Hg by means of intravenous trimethaphan (Arfonad) also showed a diminished water uptake of about the same degree as the hypothermic group. The dye uptake was also less but diminished binding was not demonstrated, nor was there definite evidence of a diminished iodinated I_{131} serum albumin uptake. The sodium and chloride changes were similar to those seen in the hypothermic group. The water uptake in the grossly normal portion of the damaged hemisphere was not suppressed and the water content of the undamaged hemisphere was greater than the control, suggesting that sustained systemic hypotension has an untoward effect on the brain. There was also an increase in potassium in these parts of the brain. This is viewed as a direct effect of the drug.

It is concluded that the edema associated with freezing lesions of the brains can be effectively treated by either sustained systemic hypothermia or hypotension but that the former is more effective than the latter. Since the state of hypothermia was associated with moderate hypotension and the state of hypotension was a mild hypothermia, the beneficial effects could not be attributed to either factor acting alone, and we were unable to define the critical levels for suppression of edema.

Agarwal, Gyan C., Berman, Bradley, M., and Stark, Lawrence: A Lumped Parameter Model of the Cerebrospinal Fluid System. *IEEE Trans. Bio-Med. Eng., 16: 45-53, Jan. 1969.*

The many mutually interacting phenomena which control cerebrospinal fluid dynamics and reflect them to other physiologic systems constitute a rather complex mechanism. The classical piecemeal approach for analysis is not suited to study the interactions of multiple parameter changes or the manner in which some evoked change in the cerebrospinal fluid system reflects itself on other parts of the mechanism.

A lumped parameter model of the cerebrospinal fluid system is developed and presented in terms of hydraulic and electrical analogies. The parameter values in these models are obtained by direct calculations using the available physiologic data and by analog simulation. The model illustrates the complex interdependence of the various variables which control the cerebrospinal fluid system. Several phenomena of neurosurgical interest are explored through the model and the controlling mechanisms in each case are pinpointed.

Coogan, Philip S., Friese, Judith, Maganini, Helen, Berardi, Ronald S., and Hass, George M.: Purification of Growth Inhibitors in Ultrafiltrate of Chick Embryo Extract. *Lab. Invest., 20: 371-376, 1969.*

A step-wise method of purification of growth inhibitor(s) in ultrafiltrate of chick embryo extract has been described. These steps included Sephadex gel and ion exchange column chromatography and organic solvent extraction, in that order. Two inhibitory fractions were isolated in solution by extractions, with acetone and absolute methanol. In both extracts the inhibitor(s) was localized to anionic ultraviolet-asborbing foci by high voltage paper electrophoresis and had identical electrophoretic mobility. Eluates of these inhibitory foci yielded singled ultraviolet-absorbing spots of identical RF in two different paper chromatography solvent systems. By concentration of the acetone extract, the residual acetone-soluble material represented a 100-fold purification by weight of the original lyophilized ultrafiltrate. This had a growth inhibitory action on chick liver "fibroblasts" in vitro, equal to that of the original ultrafiltrate. Its ultraviolet absorption maximum was a 2580 A units and did not shift at extremes of pH. Estimates indicated that it was active in concentrations of approximately 1 x 10^{-6}M. Identification of this inhibitor(s) with other inhibitors isolated from tissue extracts or derived from nucleic acids was not established. It seemed that the inhibitor(s) might belong to an obscure group of "minor" nucleic acid derivatives, which may have an important role in regulation of cell multiplication.

Kuettner, K. E., Eisenstein, R., and Robinson, J.: Lysozyme in Preosseous Cartilage. *Fed. Proc., 27: 1113, Apr. 1968.*

The concentration of lysozyme (LYS) increases threefold toward the ossification front of preosseous calf scapular cartilage. Rabbit anti-egg white LYS precipitates cartilage LYS. Fluorescent-labeled antibodies indicate that LYS is localized inside chondrocytes. Addition of LYS to organ cultures of preosseous cartilage results in a darkening of the tissue. The zone of hypertrophy, where LYS in vivo is present in highest concentration, is spared. Microradiography indicates that this opacity-effect is not due to calcification. Changes in the staining reactions of the matrix and enlargement of chondrocytes are also induced. The LYS-effect is reversible upon withdrawal, with subsequent accelerated growth. Ultraviolet irradiation of LYS resulted in an enzymatic inactivation, decrease of the absorption optimum at 2530 Å, and loss of ability to combine with specific anti-LYS antibody. Inactivated LYS also induces darkening, indicating that the physiological role of LYS may be related more to its basic protein characteristics than to its enzymatic activity, particularly since it has no known substrate in cartilage.

Kuettner, K. E., Soble, L. W., Croxen, R. L., and Eisenstein, R.: Effect of Egg White Lysozyme on Cartilage Organ Culture. *Fed. Proc. 28: 552, 1969.*

Lysozyme (LYS) is present in high concentrations in epiphyseal cartilage (CART). Addition of LYS to cultures of such CART causes altered matrix staining reactions. Its relationship to CART development was studied.

In chick embryos, LYS first appears in significant concentrations when the epiphysis develops. Until then addition of LYS to cultured bones does not induce changes in matrix staining nor does significant binding of LYS occur. After epiphysis formation, LYS concentration increases, CART binds LYS from tissue culture medium, and addition of LYS induces altered matrix staining. In mouse embryos, LYS induces similar changes and does not affect growth rate. Removal of LYS results in an apparent transitory increase in CART growth rate. Protamine, another basic protein, induces irreversible changes different from those induced by LYS including growth arrest apparently due to arrest of matrix synthesis. Fracture callus also contains large amounts of LYS in some stages of healing. These data suggest that the function of LYS is related to particular stages of CART development.

Dold, H. J. and Northrop, R. L.: Analysis of the Diluents Used for Rubella Virus Hemagglutination and Hemagglutination-Inhibition Tests. *Appl. Microbiol., 18: 221-227, Aug. 1969.*

A new diluent (ADGP) for the rubella virus hemagglutination (HA) and hemagglutination-inhibition (HI) tests is described. It was found that the HA and HI titers and the erythrocyte agglutination pattern were improved in ADGP compared to previously described diluents. The influence of the components of ADGP and of various test conditions on optimal HA and HI results were examined.

Northrop, R. L.: Effect of Puromycin and Actinomycin-D on a Persistent Mumps Virus Infection in Vitro. *J. Virology, 4: 133-140, Aug. 1969.*

Puromycin and actinomycin-D were used to treat a line of human conjunctiva cells persistently infected with mumps virus (C-M cells) in order to determine where virus synthesis is inhibited. Although 90% of the cells in C-M cultures are infected, little or no infectious virus is produced by most cells in a growing culture. Adding puromycin to inhibit protein synthesis resulted in the production of infectious virus. Thus, all the viral proteins needed for virus completion were made in the growing cells. When actinomycin-D was added to growing cells, infectious virus was again produced. Since mumps virus synthesis is actinomycin-D-insensitive, this suggested a host control of the virus. Interferon was not detected. The possible mechanisms of host control are discussed.

Shramek, Grace and Deinhardt, Friedrich: Development of an Attenuated Mumps Virus Vaccine. II. Immune Response of Animals to Vaccination with Inactivated and Live Attenuated Mumps Viruses. *J. Immun., 102: 1093-1098, 1969.*

The immunogenicity and virulence of mumps viruses for mammals decreases during adaptation to growth of the virus in avian cells. However, mumps viruses grown for more than 30 passages in embryonated hen's eggs and/or CEF cell cultures can still infect mammalian cells in culture and alter the immune status of guinea pigs by infecting them. The immune responses of guinea pigs to even the most attenuated mumps viruses are qualitatively different from the responses to inactivated mumps viruses.

Curtin, J. W. and Greeley, P. W.: Radical Surgical Approach to Chronic Suppurative Hidradenitis of the Axillary, Inguinal, Perianal, and Genital Areas. *Bull. Soc. Int. Chir. 1: 1-7, 1969.*

Suppurative hidradenitis is a benign inflammatory disease affecting the apocrine sweat glands and most commonly involves the skin and adjacent subcutaneous tissues of the axilla, inguinal, perianal, genital and submammary regions. Very little of the disease, its course and treatment are seen in the surgical literature since it has been considered a benign dermatological disease.

Our personal experiences during the past ten years with 63 consecutive cases of the chronic form of suppurative hidradenitis lead us to advocate a much more radical surgical approach than has heretofore been suggested by our medical and dermatological colleagues. Indeed, we disagree with some of our surgical brothers who advocate "piecemeal" resections in chronic cases.

Our personal conviction is that a cure of this benign but extensive disease process can only be obtained by wide and deep surgical excision of all involved areas including sinus tracts, underlying subcutaneous fat and fibrous tissue and incorporated glands.

Curtin, John W. and Holinger, Paul H.: Automobile Fractures of the Larynx and Upper Trachea: Immediate Treatment, Complications and Late Reconstructive Procedures. *Bull. Soc. Int. Chir., 1: 35-46, 1969.*

Automobile accidents are responsible for the increased frequency of laryngeal injury and in particular fractures of the larynx and/or trachea. In a previous summary of our experiences 43 of 82 laryngeal fractures were reported as having occurred in transportation vehicles.

Since 1959, 43 additional patients with severe laryngeal and tracheal trauma have been seen. Over half of these patients treated required extensive external and internal laryngeal and/or cervical tracheal reconstruction because of stenosis or atresia.

This presentation details: 1. The management of the acute injury to avoid serious complications; 2. Late reconstructive procedures, using skin graft, pedicle flap and cartilage graft support, follows the removal of deforming scar tissue contractures.

Ecanow, Bernard, Balagot, Reuben C., and Bandelin, Valerie: Effect of Suspended Particles on the Gas-Absorbing Ability of Lung Surfactants. *Amer. Rev. Resp. Dis., 99: 106-108, Jan. 1969.*

The effect of powder particles on the ability of lung surfactant to absorb a nonpolar gas, halothane, was studied by placing powdered sulfamerazine upon concentrated and dilute solutions of lung surfactant and upon solutions containing no surfactant. Samples of these solutions and of solutions containing powder were placed into a tonometer. The space above the samples contained halothane gas. The rate of gas disappearance from the space was followed by withdrawing aliquot gas samples and measuring halothane concentration by means of gas chromatography.

It was found that concentrated surfactant solution without powder absorbed the gas rapidly and to the greatest extent. Concentrated surfactant solution containing suspended powder absorbed gas to a lesser extent than solutions without powder. The rate and extent of gas absorption in both cases indicate the presence of micelles in the surface. A decrease in surfactant concentration lessened the amount of gas absorbed, and it was concluded that fewer micelles were present. The addition of powder to the dilute surfactant solution appeared to result in the concentration of surfactant upon the interface. Evidence for this was the increased absorbing ability of the suspension as compared to the solution.

Samples with an aqueous surface containing only trace amounts of surfactant absorbed little or no gas.

Andrews, Albert H. Jr., Ross, Joel C., and Osenar, Stan B.: Significance of Water in Exhaled Air. *Ann. Otol., 78: 499-511, June 1969.*

An industrial type dew point hygrometer has been used for measurement of water content in exhaled air. The procedures are fast and uncomplicated and not difficult for patients or untrained subjects.

Observations made are as follows: (1) Exhaled water content is lowest during nose breathing, higher during mouth breathing and highest during tracheal tube breathing. (2) Exhaled water content varies considerably among individuals. Regardless of breathing orifice slow-deep breathing increases the water content of exhaled air and rapid-shallow breathing decreases the water content.

Based on the above observations, the dynamic events affecting exhaled water content are as follows: 1) During inspiration the surface areas over which the air passes are cooled as the air itself is warmed and humidified. During expiration the air is cooled by its passage over these previously cooled surfaces and water condenses out in proportion to the cooling 2) Prolongation of exhalation during slow-deep breathing warms the surfaces and decreases condensation. Rapid-shallow breathing has the opposite effect. 3) Over a wide range of slow-deep and rapid-shallow breathing, the exhaled water content correlates well, but over the short span of natural breathing correlations are obscured.

The development of this concept of the dynamics of exhaled water contents enables proceeding with the clinical application of this methodology.

Leer, John A. Jr., Green, John L., Heimlich, Ernest M., Hyde, John S., Moffet, Hugh L., Young, Gordon A., and Barron, Bruce A.: Corticosteroid Treatment in Bronchiolitis. *Amer. J. Dis. Child. 117: 495-503, May 1969.*

The effect of betamethasone sodium phosphate was evaluated in the treatment of acute bronchiolitis in a double-blind study of 297 infants and young children. The results of this collaborative study, which was undertaken simultaneously in five hospitals, indicated that the corticosteroid used, and perhaps corticosteroids in general, offer little if any benefit in the general or routine treatment of acute bronchiolitis. Results were similar in patients with, or without, a family history of allergy. Although major signs were modified in more patients who received the corticosteroid than in patients in the control group, the differences between the groups were not statistically significant; these results can only be considered chance associations.

There were no detrimental corticosteroid effects in any of the patients. Compared with the patients in the control group, those treated with betamethasone sodium phosphate (3.5 mg/5 lb. over 72 hours) had a lower incidence of pulmonary infiltration, no greater need for antibiotic therapy, and no higher incidence of post-therapeutic complications. The corticosteroid neither increased the incidence of staphylococcal or other bacterial pneumonias nor masked superinfections.

The study of the natural history of the disease confirmed that bronchiolitis occurs twice as often in male infants as in female infants. Peak incidence occurred in male infants who were three months of age or less and in female infants four to six months of age.

Respiratory tract signs were more severe in the patients in this study than in those described in other reports, and fever over 100F (37.8C) was more frequent.

Other aspects of bronchiolitis did not differ substantially from those reported previously.

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